The Sternal Integument and Scent Marking in the Brushtail Possum, *Trichosurus vulpecula:* Gender and Seasonal Differences

by
Kristen L. Hynes (B. Sc. (Hons))
(Louise)

Zoology

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Abstract

The marsupial brushtail possum (*Trichosurus vulpecula)* possess a glandular area of skin over its sternum known as the "sternal gland". This region of the integument is composed of two layers: a superficial layer of holocrine sebaceous tissue and a deeper layer of sudoriferous apocrine tissue. Secretions produced by the glandular tissue are rubbed on objects by the possum as a form of scent mark. The secretions are visible as an orange to brown coloured stain on the fur covering the sternum. The structure is found in both males and females.

A review of the literature reveals that olfactory communication in the brushtail possum has been studied in some detail. In a variety of observational and experimental studies using captive and free-ranging animals information has been collected on the location and structure of scent glands, the chemical composition of secretions, the role of hormones, the range of scent marking behaviours, the response of conspecifics to odours and the possible function of odours. Despite the broad range of information already collected there are a number of aspects of olfactory communication in the brushtail possum that have not been explored in any detail, and there are many questions about the function of odours that remain unanswered.

This study aims to continue the investigation of olfactory communication in the brushtail possum by focusing on the sternal gland and examining differences between the sexes. Three main areas are explored:

- An examination of the histology and gross morphology of the sternal gland.
- Development of a method of recording sternal gland scent marking under natural conditions.
- Investigation of the use and possible function(s) of the sternal gland in the brushtail possum under natural conditions.

The first part the study is a histological examination of the sternal integument carried out using tissue from 119 males and 52 female roadkill possums collected over a period of twelve months. Canonical variate analysis was used to look for differences across gender, maturity, season and reproductive status. The histological parameters were: total glandular tissue depth, depth and percentage of holocrine sebaceous tissue and apocrine sudoriferous tissue, holocrine sebaceous and apocrine sudoriferous nuclear diameter, and apocrine sudoriferous cell height and lumen diameter. Information was also collected on changes in the amount of staining of the sternal fur during the year using roadkill animals and animals trapped in the field.

A range of differences in the histology of the sternal gland between and within the sexes, between mature and immature animals, and between groups of possums over different seasons was found.

Development of the sternal gland is related to the onset of sexual maturity. The tissue of immature males and females is not significantly different, but mature animals have significantly greater tissue development than immature animals. Among sexually mature animals, males show a higher degree of tissue development, having greater glandular tissue depths and a higher percentage of each tissue type, than females.

A number of significant seasonal differences in the histology of the sternal gland exist between the sexes and within each sex. The greatest differences between the sexes are seen during the breeding, post-breeding and dispersion periods and are related to differences in the behaviour and activity of each sex at these times. The differences were only observed in the total depth of the glandular tissue and in the holocrine sebaceous tissue parameters.

Among mature males differences in sternal gland histology are closely related to the breeding season. During the breeding season and the time leading up to breeding holocrine sebaceous glandular tissue development and activity are at their greatest. These findings are correlated with a number of physiological and behavioural changes observed in male brushtail possums during the breeding period, including increased scent marking activity, an increase in the number of chemical compounds in the secretion, increased prostate size and an increase in testosterone level. Although most of the differences among males appear to be associated with changes in holocrine sebaceous tissue there is some evidence that sudoriferous apocrine tissue parameters show increased development during the period when young are dispersing.

Among mature females seasonal differences in the size of the holocrine sebaceous nuclei were observed, with the greatest development occurring during the pre-breeding period, when females are carrying pouch young. No clear trends in sudoriferous apocrine tissue were apparent. Mature females were also examined with respect to their reproductive state. Although no significant differences were apparent, some variation in the glandular parameters is evident. Holocrine sebaceous tissue shows its greatest development in anoestrus females and its lowest development in oestrus individuals, and the depth of the sudoriferous apocrine tissue is greatest in oestrus females.

The second part of the investigation is a two-year field study conducted to examine sternal gland scent marking in the brushtail possum. This task was made difficult by the nocturnal, cryptic and partly arboreal behaviour of the species. A range of techniques (including: acoustic biotelemetry, direct observation, radio tracking, and implantable transmitters) were trialed to find a suitable method for collecting information on scent marking under natural conditions. The advantages and disadvantages, the success and limitations of each technique are discussed. The method developed and used in this study involved a combination of spool-and-line tracking and application of fluorescent pigments to the sternal region. The combination of these methods has a number of advantages. The materials required are cheap and easy to construct, and they are easily attached and do not appear to affect the behaviour of the animal. It is possible to collect data without an observer being present at the time of the activity, which has the added advantage that the behaviour of the animal is not influenced by the presence of an observer. The technique allows data to be collected on more than one animal at a time and for information on the location and size of the scent mark to be determined.

Spool-and-line tracking and fluorescent pigments were used to investigate the use and possible functions of the sternal gland scent marking in the brushtail possum. Data on the home range, use of dens, the spatial distribution of scent marks within the home range, the types of objects marked, and the timing of scent marking during the year was collected. Seasonal and gender differences were found.

Males had larger home ranges than females. Although the home ranges of males and females overlapped, within each gender there was very little overlap of home ranges. Spool-and-line tracking revealed that individuals cover large areas of their home range during one night and that most of the area within a home range is used, although some areas are used more frequently than others.

The majority of dens sites in this study were located off the ground. Dens were used in at least two different ways by possums. Dens found in hollow logs, close to the ground and in trees were often used during the night, in some cases to shelter from inclement weather. During the day den sites in trees were preferred by resting animals, with sites on the ground being used by sick or injured individuals. Between 8 to 12 den sites were recorded per individual, with dens being spread throughout the home range.

Scent marking by brushtail possums was recorded on a variety of objects including, tree trunks, branches of shrubs, clumps of grasses, fallen logs, fallen sticks, branches and bark on the ground, pieces of wood, rocks and traps. Most marks were made on objects on the

ground or close to the ground. There is no evidence of boundary marking of the home range by either sex. Some evidence of marking as a method of resource protection is evident.

Maturity, gender and seasonal differences were found in sternal gland marking. No scent marking was observed in sexually immature individuals of either sex. Among mature individuals males were observed to mark more often with the sternal gland than females. The majority of the marks made by males were deposited on or within two metres of a tree or a trap. Females made most of their marks on objects on the ground and only a third were found on or close to trees or traps. The size of scent marks on trees did not differ between sexes, although marks on all other objects were generally larger in males than females. Males performed most marking in the dispersal, pre-breeding and early part of the breeding season. Once mating had occurred and during the time females had young in the pouch sternal scent marking among males was infrequent. Rates of marking in males correlated with changes in the degree of staining of the sternal fur. Among females sternal scent marking was highest when they had young in the pouch, with a lower level occurring during the dispersal phase and during the pre-breeding and breeding seasons in oestrus females. The level of sternal staining was lower during the period of greatest sternal marking in females.

The results of the study indicate that although both male and female brushtail possums posses sternal glands there is significant sexual dimorphism in the gross morphology and histology of the sternal gland and in the deposition of secretions produced by the gland. Reasons for these differences are discussed by examining the possible functions of sternal gland odours and scent marking in the brushtail possum.

Among males there are three distinct periods of sternal gland development and scent marking behaviour. The first occurs during the pre-breeding and breeding periods and is characterised by a higher level of holocrine sebaceous tissue development and scent marking. At this time an increase in scent marking may function to familiarise females with potential mates and/or to deter rival males. The second period in males occurs during post-breeding when females have young in the pouch and is characterised by a low level of gland development and marking behaviour. If the function of odours is to attract a female or deter a rival it is reasonable to expect that marking and gland development would decrease during a period when neither of these functions is operating. The third period corresponds with the dispersal of young and is characterised by greater development of sudoriferous aporcrine elements and an increase in scent marking. Although there is no evidence that scent marks are concentrated around home range boundaries, odours deposited at this time are most likely related to protection of resources such as den trees and feeding trees.

The pattern of sternal gland development and scent marking in mature female possums is most likely related to the protection of resources. During the winter months when females are carrying pouch young an increase in marking may serve to protect trees required for shelter and food.

In both sexes resource protection appears to be the major role for olfactory communication in the brushtail possum. The mechanisms of odour function, however, are more difficult to ascertain. Possible mechanisms of odour function through the establishment of dominance hierarchies among adjacent and overlapping individuals, through "scent matching" and "competitor assessment" by potential intruders and rivals, and through "confidence boosting" and "reassurance" of a resident individual are discussed.

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Chapter 1. Introduction

1.1. Olfactory communication in mammals

The widespread existence of olfactory communication among mammals is well known (Ralls 1971; Eisenberg and Kleiman 1972; Thiessen & Rice 1976; Brown 1979). Since the publication of Schaffer's (1940) book on the distribution and structure of skin glands in mammals, numerous studies and reviews of scent organs have been written including Green (1963), Adams (1980), and Müller-Schwarze (1983). Indeed, examples of odour-producing structures have been found in species belonging to almost all the orders of mammals (Müller-Schwarze 1983). Much is also known about the range of often specialised scent marking behaviours employed by mammals to liberate odours into the environment (Ralls 1971; Mykytowycz 1972; Eisenberg and Kleiman 1972; Johnson 1973; Thiessen and Rice 1976, Brown 1979; Müller-Schwarze 1983). The effects of odours on individual behaviour and the possible social functions of odours have also been studied. A substantial body of literature based on observations and experiments conducted under natural and captive conditions exists for a range mammals (Mykytowycz 1970, 1972; Eisenberg and Kleiman 1972; Johnson 1973; Stoddart 1974; Thiessen and Rice 1976; Brown 1979; Müller-Schwarze 1983).

Odours produced by scent glands have extensive intra- and inter-specific communicatory functions. Mammals use odours in recognition, social organisation, territorial defence, alarm signalling and reproduction, (Ralls 1971; Eisenberg & Kleiman 1972; Mykytowycz 1970, 1972; Johnson 1973; Stoddart 1974, 1980a; Brown 1979; Müller-Schwarze 1983).

Recognition is a major function of odours in mammals (Shorey 1976). Odours may enable a mammal to identify other members of the species, members of the social group and even individuals. Recognition of age, sex and reproductive state and social status may also be possible. Many mammals sniff odour-producing regions of conspecifics when they meet (eg black-tailed deer sniff the tarsal glands of one another upon meeting (Müller-Schwarze 1971). Studies of the chemical composition of odorous secretions have shown that there are differences in the composition and relative amounts of compounds between social groups, families and individuals. Differences between individuals are often related to differences in sex, age, social rank, and/or reproductive status (eg the flank gland secretion of the vole, *Arvicola terrestris,* varies between juveniles and adults, and between the sexes at different times of the breeding season (Stoddart *et al* 1975). An ability to recognise individuals using odour has been shown in many species, including rabbits, *Oryctolagus cuniculus* (Goodrich & Mykytowycz 1972), pikas, *Ochotona princeps* (Meaney 1987) and Djungarian hamsters, *Phodupus campbelli* (Vasilieva & Sokolov 1994).

The ability to recognise one another is vital in the social organisation of species, as the continued cohesion of social groups relies on mutual recognition. Identification of family and social group members using odour has been demonstrated in the sugar glider, *Petaurus breviceps* (Schultze-Westrum 1965). The social structure of a group may be established and maintained through dominance hierarchies based on quantitative or qualitative differences of individual odours (Mykytowycz 1972; Shorey 1976, Brown 1979). Morphological and behavioural studies indicate that dominance is characterised by larger scent glands, greater amounts of secretion and/or a greater scent marking frequency (Shorey 1976). For example, dominant male and female marmosets, *Callithrix jacchus,* mark more frequently than same-sex subordinates of the social group (Epple 1972);

dominant male sugar gliders have greater scent production and mark more than other males (Schultze-Westrum 1965, 1969). Secretions from the anal glands of dominant male rabbits have a stronger smell than those of subordinates (Hesterman & Mykytowycz 1968). The establishment of dominance hierarchies and the recognition of the status of individual members in a social group through odour may decrease agonistic behaviour within the group (Brown 1979).

Odours may enable identification of strange individuals who may pose a threat to the stability of a social group, or to an individual. Use of odours in territorial defence, particularly as a means of repelling intruders, has long been recognised as an important function (Mykytowycz 1972; Johnson 1973; Müller-Schwarze 1983; Gorman 1984). Members of black-tailed deer social groups, for example, mark branches with their foreheads and sniff branches marked by others. Although sniffing has no obvious behavioural effects on members of the social groups, strangers who sniff a marked branch quickly retreat (Müller-Schwarze 1971). Territorial boundary recognition through olfactory cues is also seen in mice (Harrington 1976), a range of carnivores (Stoddart 1980b), lemurs (Millhollen 1986), and many other mammalian species. Scent marks are known to be deposited around the boundary of a territory or close to important resources within a home range, such as food and den sites. Odours may also be used by members of a social group to alert or warn conspecifics of possible threats to their safety. These so-called alarm signals are produced in situations of fear or stress and may result in conspecifics retreating from, or avoiding, areas where the odours are deposited. Black-tailed deer, for example, release scent from their metatarsal glands when chased or released into a strange environment. Other deer show avoidance of the scent and cease activities such as feeding (Müller-Schwarze 1971). Avoidance behaviour may also be elicited by interspecific odours, particularly those of predators. Mountain beavers *(Aplodontia rufa)*, for example, feed less in the presence of odours from mink, bobcats and coyotes (Epple *et al* 1993).

Marking of the environment with scent may have a role in boosting the confidence of and reassuring the markers as they move around their home range (Ewer 1968; Mykytowycz 1972, 1974; Johnson 1973; Shorey 1973; Brown 1979; Müller-Schwarze 1983). Mykytowycz 1972 (cited in Mykytowycz 1972 — unobtainable symposium paper) states that "only within an area where its own odour prevails will an animal behave freely and participate in breeding activities". Support for this comes from observations and experiments that show resident individuals are consistently more successful than intruders during agonistic encounters (Eisenberg & Kleiman 1972). This appears to be related to the enhanced self-confidence of animals when on familiar ground, and the corresponding decreased self-confidence for individuals on unfamiliar ground. It has also been observed that during agonistic encounters animals will lick their own glandular regions (eg male red kangaroos, *Megaleia rufa* (Sharman & Calaby 1964)). Further evidence comes from the high level of marking activity in response to novel objects, new environments, or following the removal of scent from a familiar habitat, seen in many mammals (Johnson 1973). Closely related to the reassurance role of odours is the concept that odours are used by animals for orientation within their habitat (Johnson 1973; Shorey 1976; Müller-Schwarze 1983; Benhamou 1989). Evidence for this function is seen in beaver *(Castor canadensis)* (Müller-Schwarze & Heckman 1980). It had been suggested that scent mounds located on trails some distance from a beaver's lodge aid in the orientation of resident beavers.

Odours play an important function in reproduction in mammals and may be involved at all stages of the reproductive process including attraction of a mate, advertisement and recognition of reproductive status, induction of oestrus and lordosis, courtship, ovulation, pregnancy, mother-young relationship, and sexual maturation (Stoddart 1980a & b). Evidence for the role of odours in reproduction is provided by three observations. Firstly, in many mammals, scent glands, secretion and marking behaviours develop as the animal reaches sexual maturity (Johnson 1973; Brown 1979; Stoddart 1980b). For example, many of the odour producing glands in rabbits develop as the animal attains sexual maturity (Mykytowycz 1965, 1966a & b). Secondly, some scent glands show increased activity or

may only be functional during the breeding season (Mykytowycz 1972; Johnson 1973; Stoddart 1980b). In the bandicoot, *Isoodon obesulus*, the subauricular gland in both sexes is largest during the breeding season (Stoddart 1980c); the forehead gland in white-tailed deer *(Odocoileus virginianus)* shows increased activity (moderate in females and very high in males) during the rut (Atkeson & Marchinton 1982). And thirdly, many glandular structures and scent marking behaviours are sexually dimorphic, often occurring more often in males than females (Mykytowycz 1972; Johnson 1973; Stoddart 1974, 1980b; Thiessen & Rice 1976). Male sugar gliders possess frontal and gular scent glands that are not found in females (Schultz-Westrum 1965, 1969; Stoddart & Bradley 1991); in capybaras *(Hydrochaerus hydrochaeris)* the morillo glands is highly developed in males, but generally invisible in females (MacDonald, Krantz & Alpin 1984); the sternal gland of male North American opossums *(Didelphis virginiana)* shows greater morphological and histological development and secretory activity than females (Meisner 1986).

To be able to adequately interpret the functions of odours in the life of an animal, a range of information is desirable. Firstly, a broad understanding of the social organisation and ecology of the species is important. This encompasses knowledge about its life-cycle, breeding, mating, longevity, care of offspring, dispersion of young, movement of young and adults, spacing, use of resources (eg food, shelter, nests), interactions between conspecifics, and hierarchical relationships. Secondly, information about the types of scent organs, the types of marking, the timing, frequency, distribution and location of marks, the context of marking, and the response of conspecifics to marks is needed. Differences and changes in the gross morphology, histology, hormonal control and chemical composition of the scent organs, and their secretions between individuals of different genders, age and status, and at different times of the year is also important.

Despite recognition of the importance of olfaction in the behaviour and social organisation of mammals, there are very few species in which knowledge from ecological and behavioural studies has been integrated with information about scent organs and scent marking behaviour in such a way that the functions of odours produced by the species is well understood. Some species in which the relationship has be explored in greater detail include the rabbit, *Oryctolagus cuniculus* (Hesterman & Mykytowycz 1968; Mykytowycz 1958, 1959, 1965, 1966a,b & c, 1968; Mykytowycz & Dudzinski 1966; Mykytowycz & Gamble 1969; Mykytowycz & Hesterman 1970; Mykytowycz & Rowley 1958), golden hamsters, *Mesocricetus auratus* (Johnston 1975a, b, c, 1993; Johnston & Rasmussen 1984; Johnston & Robinson 1993; Johnston *et al* 1993; Johnston *et al* 1994; Johnston, *et al* 1995; Wilcox & Johnston 1995), black tailed deer, *Odocoileus hemionus columbianus* (Quay & Müller-Schwarze 1970; Müller-Schwarze 1971), the brushtail possum, *Trichosurus vulpecula* (Winter 1977; Biggins 1979; Salamon 1994, 1998), and the sugar-glider, *Petaurus breviceps* (Schultz-Westrum 1965, 1969; Stoddart & Bradley 1991; Bradley & Stoddart 1992, 1993; Stoddart, Bradley & Hynes 1992; Stoddart, Bradley & Mallick 1994; Mallick *et al* 1994). The sparsity of species studied in detail is probably due in part to the comparatively recent interest in chemical communication as an important part of mammalian behaviour and social organisation.

1.2. The common brushtail possum

The Common Brusthtail Possum, *Trichosurus vulpecula* Kerr, 1792 (Marsupialia: Phalangeridae) has been well studied in both the field and laboratory. A nocturnal, solitary, arboreal folivore, it is the most widespread and abundant of the possum species in Australia (Troughton 1941; How 1974, 1983; Flannery 1994). It is found in eastern Australia from Cape York (excluding the most northern part) to Tasmania, south-west Western Australia, south-eastern South Australia and wooded areas of central Australia. Three sub-species have been recognised: *T. vulpecula vulpecula* in south-eastern and south-western Australia, *T. vulpecula johnstonii* in northern Queensland, and *T. vulpecula fuliginosus* in Tasmania (How 1974), although there is some dispute over this classification. A recent genetic study (Triggs 1990) does not support the subsepcific status of *T. vulpecula fuliginosus,* since the divergence between Tasmanian and mainland populations is no greater than that between mainland populations. Further, Flannery (1994) suggests that *T. vulpecula johnstonii* be considered a separate species, *T. johnstonii*, based on morphological differences and possible reproductive isolation, despite the existence of sympatric populations. Using morphological characters, karyotypes, electrophoretic allozyme and ecological data, Kerle, McKay and Sharman (1991) concluded that there were insufficient differences between populations to warrant division into more than one species. The geographic separation of Tasmanian brushtails, however, led them to support the retention of *T.v. fuliginosus* as a subspecies.

Across its range the size and colouration of the brushtail possum is highly variable (Tyndale-Biscoe 1973). Body weights range from 1000g in northern Australia to greater than 4000g in Tasmania (Hocking 1981). Males are generally larger than females, with mature body weights usually between 2000-4000g for males and 1500-3500g for females (Barnett, How & Humphreys 1979; How 1983; Flannery 1994). Over most of its range the dorsal fur is a silver-grey colour, the ventral surface white to pale grey, and the tail black. In northern Queensland there is a short-haired, copper-coloured form; in Tasmania the fur is woollier and colour ranges from grey through black, to a rufous-coloured form (How 1983).

The brushtail possum inhabits most areas were there are trees, particularly woodlands and open forest. Its distribution appears not to have been detrimentally effected by European settlement with high densities being found in urban areas (Jones 1924; How 1983; Flannery 1994). A nocturnal species, it utilises hollows in tree branches and trunks, and fallen logs, as den sites during the day; and in suburban areas, man-made structures, particularly roof spaces, are commonly used. The brushtail possum spends much of its time on the ground, although it is an arboreal species possessing sharp claws, an opposable first toe on the hindfoot and a prehensile tail, which it uses to climb.

The diet of the possum is varied (Freeland & Winter 1975; Fitzgerald 1984; Statham 1984) and includes leaves (including *Eucalyptus* and *Acacia* spp), flowers, fruit, buds, bark, grasses, herbs, clover and other pasture plants, and occasionally insects (see Kerle 1984). Possums are unable to eat a diet consisting of eucalypt leaves only, due to the high levels of toxic volatile oils and phenols and their inability to completely detoxify theses compounds (Freeland & Winter 1975).

In most of its range the common brushtail possum has a major breeding season in autumn and a minor one in spring, although births have been recorded in all months of the year (Bolliger 1940; Tyndale-Biscoe 1955; Dunnet 1956, 1964; Gilmore 1969; Crawley 1973; Kean 1975). Non-permanent pairs may be formed for between 30-50 days before mating (Winter 1977), although the species is polygamous (How 1972; Winter 1977). Even though a male may exhibit consort behaviour towards a female, he may leave her for a time to mate with another female who comes into oestrus. Males may also form a second consort relationship during a breeding season after mating with the first consort. Similarly, a

female, whether accompanied by a male for an extended period of time or not, may mate with a number of males during the same period of oestrus (Winter 1977).

Sexual maturity in both sexes is reached during their second year (Tyndale-Biscoe 1955; Pilton & Sharman 1962; Gilmore 1969). Females frequently begin oestrus cycling at 12 months of age (Kean *et al* 1964; Smith *et al* 1969) and are parous by the end of their second year (Smith, Brown & Frith 1969). Pilton and Sharman (1962) recorded an example of a female having her first oestrus at nine months. Females show seasonal changes in fertility. During the summer months the majority of females undergo a period of anoestrus (Tyndale-Biscoe 1955; Gilmore 1969); in the main autumn breeding season most mature females come into oestrus, and a second period of oestrus occurs in some females in the secondary spring breeding season (Tyndale-Biscoe 1955; Pilton & Sharman 1962; Gilmore 1969). In males maturity is reached between 18 and 24 months of age (Gilmore 1969; How 1972) when the testes undergo rapid growth and become spermous (Tyndale-Biscoe 1955; Gilmore 1969). Upon reaching sexual maturity there is no seasonal variation in testes size and spermatogenesis occurs throughout the year (Bolliger & Carrodus 1938a; Bolliger 1942, 1946; Tyndale-Biscoe 1955; Dunnet 1956; Gilmore 1969). The prostate, however, shows marked seasonal variation, with the greatest mean weights being recorded during the two times of the year when females are in oestrus (Gilmore 1969).

In most populations, over 90% of the females breed once a year and in some areas up to 50% of females may breed in both seasons (Bolliger 1940; Dunnet 1964; How 1983). The oestrus cycle is 32 days long between April and June and 26 days long between June and December and gestation lasts 17.5 days (Pilton & Sharman 1962). Females are polyoestrus and monovular (Pilton & Sharman 1962). A single young is usually born, although twin embryos (Tyndale-Biscoe 1955) and births have been recorded (Clout 1977). The young spends the 4-5 months after birth attached to one of two teats in its mother's pouch where it develops rapidly. Once its eyes are open and it is furred, the young spends a further 1-2 months suckling and riding on its mother's back before being weaned (Dunnet 1956, 1964; How 1972). The age of independence of young from their mother appears to be highly variable. Dunnet (1956) reported capturing young without their mothers for the first time between 150 and 178 days. How (1972) and Hocking (1981) found that young less than 200 days old were generally not independent, although maternal dependence decreases after 175 days.

Mortality in the pouch is considered to be low (Dunnet 1964; How 1972). However, once young leave their mother mortality increases, particularly among males (Dunnet 1964; How 1972; Winter 1977). Following dispersal, How (1972) found only 24.5% of males survived to two years of age, compared to 35.5% for females. This bias in the survival rate for the first two years of life is reflected in the disproportionate sex ratio of the adults (ie $\Xi < X$) found in some populations (Dunnet 1964; How 1972). The low juvenile survival rate is believed to be the result of an inability of young possums to become established in habitats that are already fully utilised (How 1972). Winter (1977) found that dispersion was centred around den sites and that without the protection offered by a den it is probably difficult for an individual to establish a home range, particularly in open habitats. Possums that become established and survive are known to live up to twelve years in natural populations (Crawley 1970).

The difference in survival of juvenile males and females is probably partly related to differences in the pattern of dispersal of the young. Juvenile males tend to disperse further from the maternal range than females (Dunnet 1964; Winter 1977; Ward 1985). This may be due in part to resident males excluding young males from occupied home ranges (Dunnet 1964). Few aggressive encounters between resident adult males and juveniles males raised in the same area were recorded by Winter (1977), although most encounters between resident adult males and young males of unknown origins were aggressive in nature. Despite the lack aggression by adult males to young males born in their home ranges Winter proposed that adult male aggression was partly, if not wholly responsible for the dispersion of juvenile males. Adult males did not appear to influence the dispersal of juvenile females; adult male behaviour towards a young female is neutral until she first

comes into oestrus and begins to mature sexually (Winter 1977). Juvenile females are more likely to be recruited into the population, establishing home ranges within or adjacent to their maternal home range (Dunnet 1964; Winter 1977). Clout and Efford (1984) found that 59.1% of the males recruited into a population were of unknown origin, whereas 71.6% of the females recruited were from the local population.

The brushtail possum is generally solitary. The only long term contact occurs between a female and her offspring (Tyndale-Biscoe 1973); shorter periods of contact between adult males and females occur during consort relationships prior to breeding (Winter 1977).

Dunnet (1964) suggested that there are probably two types of possums — those that are resident in an area and occupy a stable, discrete home range, and transient individuals (usually immature and male) that do not possess or occupy a definite area. Winter (1977) recognised two classes of males: younger adult males which do not have established home ranges and older established males. The older males are dominant to the younger ones. Dominance hierarchies correlated with age and/or size also exist among females (Winter 1977).

Among resident animals there are differences in the characteristics of the home ranges between the sexes. Males generally have larger ranges than females (Dunnet 1956, 1964; How 1972; Jolly 1973 Crawley 1973; Winter 1977; Hocking 1981). The size of the ranges reported varies greatly and is detailed in Table 1.

Table 1. Reported mean home range size (ha) of male and female brushtail possums.

♂	♀	Study site	Method of assessing home range size	Data source
3.0 (7.49 acres) "resident males"	1.1 (2.67 acres) "resident females"	Canberra (Australia): highly modified bush and pasture	traps & observations	Dunnet (1956)
7.41	4.67	North-eastern NSW (Australia): woodland and pine plantation	traps	How (1972)
0.8	0.3	Banks Peninsula (New Zealand): mixed bush, scrub and pasture	traps & observations	Jolly (1973)
0.81	0.46	Orongorongo Valley (New Zealand): indigenous forest	traps	Crawley (1973)
3.71	1.74	Moggill, Queensland (Australia): modified "grassy open forest"	all night observations	Winter (1977)
up to 9	up to 6	Southern Tasmania (Australia): Commercial hardwood forests at various stages of regeneration	traps	Hocking (1981)

It has been suggested that not only are there differences in the size of ranges between the sexes, but that males and females "occupy" their ranges differently. Dunnet (1964) concluded from his study that males occupied mutually exclusive areas that are generally defended and could therefore be regarded as territories. Other studies, however, do not concur with Dunnet. Crawley (1973) found extensive overlap between male ranges and did not observe any conspicuous territorial behaviour. A closer examination of Dunnet's data by How (1972) revealed that male ranges were not mutually exclusive and that nearly all overlapped with other males. Winter (1977) did not consider males to be completely territorial and found that their home ranges did overlap. The only evidence of territorial behaviour was observed at den sites, and here females were always dominant to males.

Beyond den sites no evidence of patrolling or scent marking of boundaries was found (Winter 1977).

The home ranges of females generally overlap to a greater or lesser degree (Dunnet 1956, 1964; Crawley 1973; Winter 1977) and may overlap completely with other females (Dunnet 1956, 1964). Winter (1977) found that in areas where dens were well spaced the home ranges of females did not overlap.

Home ranges of males and females have been shown to overlap (Dunnet 1964; Winter 1977). Male ranges often have one to two females overlapping, although reports of up to eleven females overlapping with one male have been reported (Dunnet 1964). Within both male and female ranges intrasexually exclusive areas exist, usually in the immediate vicinity of the den tree (Winter 1977). Although possums are considered to be fairly sedentary there is evidence that they make "occasional sallies" out of their usual home range (Dunnet 1956; How 1972; Winter 1977). Winter (1977) observed that males following oestrus females would venture out of their normal home range.

The variation in range size and differences in the amount of overlap between conspecifics of the same sex reported in different studies is probably partly a reflection of differences in the habitats and the population density at each site. In areas where the habitat is homogenous home range size varies little over the seasons. In more heterogeneous areas there is much seasonal variation in the use of areas within the home range (Hocking 1981), with the availability of food sources influencing home range use (Jolly 1973).

1.3. Olfactory communication in the brushtail possum

Brushtail possums use two main methods of communication, vocalisation and olfaction (Biggins 1984) —both of which are probably involved in determining and maintaining the spatial separation of individuals. Eighteen distinct vocalisations by the brushtail possum are known (Winter 1977). They are used in a variety of contexts, with most occurring during face-to-face interactions. Two distinct functional categories are recognised: those aimed at causing the withdrawal of another individual (ie threat vocalisations) and those which invite another individual to approach (Winter 1977). During agonistic encounters between individuals deep, guttural coughs, growls and grunts, and sharp hisses and screeches are used. When startled or stressed a clicking noise is made. Chattering sounds are thought to function as alarm calls. Squeaking, zook-zook and zick-zick distress sounds are made by juveniles when they become separated from their mother. Agonistic encounters between adults males and females during the breeding season are thought to be reduced by males using a vocalisation that resembles the 'lost' call of a juvenile (Winter 1977).

The following sections are a review of current knowledge about scent glands and olfactory communication in the brushtail possum.

1.3.1. Scent glands and scent marking

Olfactory communication in the brushtail possums is mediated through a large number of specialised glandular areas that produce odoriferous secretions, including ear, chin, labial, salivary, sternal, pouch, proctodael, circumanal, paracloacal (oil and cell-secreting), interdigital and tail glands (Biggins 1984; Russell 1984, 1985). The following is a description of the type of gland found at each location and the methods employed by possums to deposit secretions produced by the glands. All the glands, except the pouch glands, are found in both sexes. The sternal gland (Bolliger & Hardy 1944; Green 1963;) and the paracloacal glands (Bolliger & Whitten 1948; Thomson & Pears 1962; Green 1963) are generally larger in males than females; and scent marking using these glands is performed more often by males than females (Winter 1977).

Ear glands
The skin covering the cartilaginous processes of the external auditory meatus is covered in hairs which are associated with large accumulations of apocrine and sebaceous glands (Green 1963). No information about the chemical composition or the mechanism used to deposit secretions from this area exists in the literature.

Chin glands
Winter (1977), citing Freeland (pers. comm), reported that there is no external evidence of a glandular region on the chin, apart from the dark brown staining of the fur under the lower jaw. Histologically, however, the lower margin of the chin, particularly in adult males, has well-developed sebaceous glands. Observations by Winter (1977) reveal that secretions from the chin are probably deposited on the substrate by possums using a behaviour known as "chinning", in which the anterior part of the mandible is rubbed on the substrate with a forward and slightly rotating movement of the head. Chinning varies from a single, short-duration, light wipe across the substrate with only a slight rotation of the

head, to a vigorous repeated rubbing with maximum head rotation. In the more vigorous chinning actions the lower lip may be rolled back and salivary and labial gland secretions may be deposited on the substrate.

Winter (1977) observed chinning on fallen logs, bare earth, tufts of grass, tree bases, branches (particularly thin vertical ones), the ends of broken branches, the rims of den entrances. Chinning may be performed alone or before a chesting movement (see sternal gland section below), or as part of a chesting-chinning combination. Some temporal patterns in chinning by males possums have been recorded (there was insufficient data for females) (Winter 1977). Although no nightly pattern in chinning was observed, seasonal variation did occur. Males exhibited a pronounced peak in February with very little chinning occurring between September and January.

Labial and salivary glands

The inner surfaces of the posterior aspects of the upper and lower lips are lined with glandular filamentous projections that produce a complex mixture of lipids (Biggins 1979). Scent marking with the labial glands occurs in the more vigorous chinning action described above. As the possum drags the side of its mouth over the substrate this causes the lips and *annulus orisi* to fold outward resulting in the filamentous projections making direct contact with the substrate. This "labial sliding" action results in saliva as well as secretions from the labial glands being deposited on the substrate (Biggins 1979).

Possum have also been observed to lick and gnaw objects in their environment (Biggins 1979; Winter 1977). It is possible that these behaviours have a role in chemoreception (using the vomeronasal organ), as they were often performed in areas that had been previously marked. Licking and gnawing may also be forms of scent marking, as copious amount of salivary and labial gland secretions are often deposited. Due to the difficultly in distinguishing the function of these behaviours, Biggins defined licking and gnawing as forms of passive marking.

Saliva, used in washing and grooming of the fur, may also have a secondary self-anointing function (Biggins 1984).

Sternal gland

The sternal gland of the possum is visible as an area of stained fur along the sternum (Bolliger 1944a, 1944b; Bolliger & Hardy 1944; Green 1963). It is composed of holocrine sebaceous and sudoriferous apocrine glands which are much larger and more active than those on the general body surface. The region produces moist, frequently copious secretions that are deposited on the substrate in a forward rubbing movement known as "chesting" or "sternal rubbing". The possum adopts a bent leg stance and lifts its chin away from the substrate, which enables the chest to come in contact with the substrate. In a forward motion the chest is rubbed up the substrate and then lifted off. The action may be repeated a number of times (Winter 1977). Winter (1977) observed that tree trunks, branches, fallen logs, bare earth and tufts of grass were marked by chesting.

As possums become sexually mature the area of brown fur on the sternum becomes increasing obvious, particularly in males. In males, secretions from the gland make the fur oily, particularly during the breeding season. In females development is slower and not to the same degree; the fur on the sternum appears almost dry in the breeding season, but becomes moist 2-3 months after the young is born (Bolliger and Hardy 1944). Winter (1977) first observed chesting and chinning behaviour in juvenile females as young as 8 ½ months and in juvenile males at 21 months, although he states that the difference may be due to inadequate observations of juvenile males. Juvenile males, however, were not observed to mark while living in the maternal home range.

Temporal patterns in chesting were observed by Winter (1977) in male possums. During the night there was a marked peak early in the evening. This consisted mainly of tree base marking. A smaller peak occurred at dawn and consisted mainly of marks made on branches of the den tree as the male returned to the den. A seasonal pattern in chesting was also observed. A distinct peak was seen in March, with other peaks in June and September. Very little marking using the sternal gland was seen between October and December.

Among captive male possums dominant individuals mark more using the sternal gland than subordinates do (Biggins 1979). Dominant males also scent marked more frequently and marked their cages more extensively than subordinates.

Pouch

The upper dermal layer of pouch skin contains sebaceous glands that are associated with coarse hairs that extend deep into the dermis (Green 1963). Pigmentation of the interior of the pouch in mature females was noted by Bolliger & Carrodus (1938b). This is first evident in some sexually immature females just before their first breeding season. No specialised marking behaviour associated with the pouch has been recorded.

Glands of the anal region — proctodeal, circumanal, paracloacal

The occurrence of proctodeal and circumanal glands has been recorded in reviews by Biggins (1984) and Russell (1984, 1985) — no details about their structure, secretions or method of odour deposition is provided.

Two types of paracloacal glands (Bolliger & Whitten 1948; Thomson & Pears 1962; Green 1963) are found in brushtail possums. The first are a large pair of "oil-secreting" glands which produce an odorous, cream-coloured, oily apocrine secretion. Biggins (1979) observed that secretions from the oil-secreting glands were deposited using cloacal dragging and were present in urine voided in normal eliminatory activities. Frightened individuals, such as those severely losing an agonistic encounter, release copious amounts of secretion onto the fur around the cloaca while adopting a submissive posture (Thomson & Pears 1962; Kean 1967; Biggins 1979). The second type of paracloacal glands are known as "cell secreting glands". They are bi- or tri-lobed in structure (Bolliger & Whitten 1948; Thomson & Pears 1962; Kean 1967), and produce a holocrine secretion that is distributed continuously in the urine (Bolliger & Whitten 1948). Apart from normal eliminatory activities, urine is deposited deliberately by possums using two techniques (Kean 1967): urine-dribbling in which the possum urinates large amounts while moving forwards in a sigmoid pattern, and urine-dripping in which urine is dripped slowly from the long vibrissae surrounding the cloaca. Secretions from the cell-producing paracloacal glands may be deposited during these urine-marking behaviours. There is no discernible odour, at least to the human nose, from secretions produced by the cell-secreting glands (Bolliger & Whitten 1948; Thomson & Pears 1962).

In captive possums Biggins (1979) observed two forms of marking involving secretions from the paracloacal glands: urine-dripping and cloacal-dragging. Urine-dripping is the same behaviour reported by Kean (1967), which he also called urine-dripping. Cloacal-dragging refers to a behaviour in which the cloaca is protruded and lowered onto the substrate as the possum moves forward depositing secretions from the paracloacal gland. Urine-dripping and cloacal dragging were defined as dispersive forms of marking by Biggins, as opposed to active or passive forms of marking: both behaviours involved the deposition of secretions over large areas rather than defined localities (as seen in chinning and sternal rubbing). Among captive male possums, Biggins (1979) observed that subordinate males engaged in more dispersive forms of marking than dominant individuals.

The most common method of distribution of oil- and cell-producing paracloacal secretions is probably by passive deposition with urine and faeces during normal eliminatory activities, as reported by Bolliger and Whitten (1948) and Biggins (1979). Observations by

Winter (1977) revealed that urine-dribbling, cloacal dragging and paracloacal gland evacuation are rare events in free-ranging possums. In 1160 hours of spotlighting observations over four years Winter only observed urine-dribbling 17 times; active marking with the white oily paracloacal secretion was never seen in the field.
No temporal, nightly or seasonal, patterns in scent marking involving paracloacal gland secretions have been identified.

Interdigital and tail glands
The occurrence of these glands has been recorded in reviews by Biggins (1984) and Russell (1984, 1985) — no details about their structure, secretions or method of odour deposition is provided

As well as scent depositing behaviours, Winter (1977) observed two kinds of sniffing behaviour in possums —substrate sniffing and air sniffing. Winter considered that sniffing indicated a possum was engaging in olfactory investigation of its environment, and that any subsequent behaviour that could be correlated with the sniffing could be inferred as a response to a scent. Air sniffing was rarely recorded and, as it was difficult to determine the source of the odour being sniffed, no data were presented. A total of 279 observations of substrate sniffing over 4 years were recorded. Sniffing was recorded as occurring on the ground, at tree bases and in trees (either on the trunk or in the branches): tree bases were the most common site sniffed. Most sniffing was done by adult males, with the major source of scent being oestrus females.

1.3.2. Chemical composition of secretions

The chemical nature of secretions from the sternal, paracloacal and labial glands has been investigated in a number of studies (Bolliger & Hardy 1944; Bolliger & Whitten 1948; Biggins 1979; Woolhouse, Weston & Hamilton 1994; Salamon 1994, 1998).

In an early study, Bolliger and Hardy (1944) found yellow rod-shaped crystals in the lumens of the sudoriferous apocrine tissue of the sternal gland, which they believed were urates. The crystals were found in the lumens of male tissue only. Secretions from the sternal gland, obtained following injection with pilocarpine, where found to contain chromogen. Chromogen was also found in secretions taken from the pouch.

Examination of the paracloacal glands by Bolliger and Whitten (1948) pointed to the probable presence of cholesterol or cholesterol esters in the oil-producing gland secretions. Cells from the cell-producing glands are secreted almost continuously and are washed away from the cloacal walls by the flow of urine. The white precipitate containing phosphates, carbonates, oxalates, spherical refractile bodies, and spermatozoa in males, that appears in the urine upon standing (Bolliger & Carrodus 1938a; Bolliger & Whitten 1940) is composed of cells produced by the cell-secreting paracloacal glands (Bolliger & Whitten 1948).

Using thin-layer chromatography, Biggins (1979) study of male possums found most classes of polar and non-polar lipids present in secretions from the sternal, paracloacal and labial glands. Extracts from the paracloacal glands contained a wider range of both types of lipids than the sternal gland. Analysis of urine samples revealed a mixture of mainly polar lipid classes that were also present in the secretions of both paracloacal glands, which is to be expected given that secretions from both paracloacal glands are expelled in the urine. The major non-polar lipids in the paracloacal glands were free fatty acids and cholesteryl esters; the sternal and labial glands contained cholesterol, cholesteryl esters,

diglycerides and triglycerides. Polar lipids extracted from the sternal, paracloacal and labial glands belonged mainly to the less polar classes and possibly the glycolipids.

Differences between individual animals in the number of lipid classes and their relative proportions were found in the glandular secretions. These were most pronounced in the paracloacal gland secretions.

Gas chromatography was used to extract a variety of other chemical compounds. The range of chemical compounds from each type of paracloacal gland did not differ much, although, the relative concentration of the compounds varied, with the oil-secreting glands containing more volatile components than the cell-secreting ones. Neither type of paracloacal gland had high concentrations of volatile, lower boiling-point compounds, however. Secretions from the sternal gland were found to contain higher concentrations of volatile substances compared to secretions from labial and paracloacal glands of the same individual.

Biggins (1979) found differences in chemical compositions between animals of different sex, age and status. It should be noted that information on the time of year and reproductive state of the adults examined was not given. Sex differences were greatest in lipids extracted from sternal gland hair samples. Adults males had high concentrations of two volatile compounds that were absent from samples from females. The chemical constituents from sternal hairs from a subadult male (14 months) were more similar to the adult male than the adult female, although secretion from a juvenile male (9 months) more closely resembled extracts from the adult female. No juvenile or subadult females were examined. Secretions from the oil-secreting paracloacal glands of adult males and females contained similar mixtures of compounds, but the relative concentrations differed markedly: higher concentrations of low boiling point, more volatile compounds were found in adult females, whereas males had higher concentrations of less volatile compounds. The chemical constituents of the subadult male and juvenile male secretions were more similar to the adult male than the adult female secretion. The cell-secreting paracloacal glands of the adults also showed differences: adult males had a greater variety of compounds than females and these occurred in higher concentrations. Secretions from the subadult and juvenile males differed from the adult male, but were more similar in structure to the adult male than the adult female. Differences in labial gland secretions between adult males and females were also found. Juvenile male secretions were more similar to adult female than male secretions and subadult male secretions more closely resembled adult male than female secretions.

Investigation of the secretions of a socially stressed male revealed a number of differences compared to other individuals studied. Secretions from both types of paracloacal glands contained relatively lower concentrations of less volatile substance than "normal" adult males. However, relatively high concentrations of several major low boiling point compounds not found in "normal" males were found in the stressed male's secretions. The sternal gland secretions of the stressed individual lacked several of the more volatile compound found in other males. Therefore, secretions of the paracloacal glands were more volatile and the secretions of the sternal gland less volatile in the stressed male than in normal males. There were negligible differences found in the secretions from the labial glands.

The chemical composition of secretions from the sternal and two types of paracloacal glands have also been investigated by Woolhouse *et al* (1994)[1]. They found little variation in the composition and relative abundance of lipid components between secretions from the pigmented hair of the sternal region and secretions from non-pigmented hairs from the chest region. The lipid classes found in the sternal and two types of paracloacal glands were distinctly different and it was possible to differentiate between the glands using the profile of fatty acids obtained from each. No differences between males and females in the lipids contained in secretions from either the sternal or either type of paracloacal glands was found.

Salamon (1994, 1998) used gas chromatography-mass spectrometry analysis to identify compounds in the sternal gland secretion of possums. Individual, gender, seasonal and dietary differences were examined in wild and captive individuals. A total of 48 different compounds were identified. The major compounds were unbranched saturated fatty acids, aromatic acids, phenols and oxygen heterocycles. Long chain alcohols and hydrocarbons were also detected. Secretions taken from the sternal gland were found to be different from those collected from the belly and back of possums.

Salamon found differences in the number and type of compounds between individuals of the same sex. Sex differences were found, even though all the compounds found in males were detected at least once in females. Generally, the number of compounds in male secretions was significantly higher than in females. There were also differences in the types of compounds generally found in secretions from each sex, with some volatile compounds (eg aromatic acids, phenols and oxygen heterocycles) usually found only in males. Seasonal differences were found in the range and abundance of chemical present. Nonanal was found in all samples of both sexes and in all months of the year, but it was most abundant in November. For free-ranging females the greatest number of compounds was found in secretions collected in November, although this finding should be treated with care, as data were not available for all months of the year. In captive females the greatest number of compounds were found in March, August, September and November. For males sampled in the field the greatest number of compounds were found in secretions in April and May, closely followed by September in one year and October in another. For captive males the secretions with the highest number of compounds were recorded in August of one year and January, June and November of the following year. Analysis of the relative amounts of each compound detected each month was undertaken for one male in captivity: in November 50% of the compounds chosen for examination showed their highest concentrations; high concentrations of some compounds were found in February and March, and; June and August showed the lowest concentrations of compounds.

1.3.3. Role of sex hormones

The effect of sex hormones, particularly testosterone, on the development and control of scent organs and their secretions is well known (Ebling 1963; Strauss & Ebling 1970). Investigations of the interaction of sex hormones and scent glands in the brushtail possum has been examined by Bolliger (1944a & b) and Biggins (1979).

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[1] NB.
An error is present in the paper by Woolhouse, Weston and Hamilton (1994) and has been perpetuated by Salamon (1998). They have incorrectly labelled the types of secretion produced by the two types of paracloacal glands. The secretion of the oil-producing glands is apocrine in nature, not holocrine. Secretory cells which line the walls of the gland swell and form round fatty globules which become the secretory product (Green 1963). The cell-producing gland secretion is holocrine, not apocrine. It consists of whole, non-liquified cells suspended in a small volume of aqueous medium which have been cast off the epithelial layer (Bolliger & Whitten 1948; Green 1963).

Bolliger (1944a, b & c) conducted a series of castration experiments to demonstrate the role of testosterone in the formation of the brown fur of the sternal region. Castration of adolescent male possums (6 months) largely prevented formation of the distinct brown patch over the sternum as the animal matured; the majority of the sternal hairs remained a grey colour. One male was administered stilboestrol before castration and oestradiol dipropionate following castration: this resulted in the transformation of the scrotal tissue into a pouch-like structure and development of only a small area of yellow-brown hairs in the sternal region. Administration of testosterone propionate to male castrates resulted in the sternal hairs developing the typical brown colour over an area equivalent to the seen in normal males of the same age. Six months after cessation of testosterone injections the brown colour of the sternal hairs had almost completely disappeared.

Removal of the ovaries of adolescent females (8 to 11 months) also hindered development of the "sternal streak"; the grey hair of the immature females did not change into the brown hair characteristic of mature females during 10months of observation following ovarectomy. Administration of oestradiol dipropionate one month after ovarectomy did not result in the appearance of more brown hairs, nor did it in cause the few brown hairs already present to become more deeply stained. There was, however, an increase in the secretion and staining of the skin around the pouch. Administration of testosterone propionate resulted in the development of a coloured area similar in size and colour to adult males.

Castration of an adult male and subsequent administration of oestrogen resulted in the sternal streak decreasing in size by approximately one-third and changing from a reddish-brown colour to a yellow-brown colour. Ovarectomised adult females became obese, but no marked differences in the sternal streak were observed. The only noticeable change was that the sternal streak was more yellow and drier compared to control females.

From these studies Bolliger concluded that the brown sternal hairs of the possum are mainly a male sex characteristic, and that the activity of the glands of the sternal region and their production of chromogen (which gives the hair its brown colour) is mainly under the control of testosterone.

Biggins (1979) conducted a series of experiments examining the effect of castration and administration of sex hormones on scent marking behaviour, agonistic behaviours and scent gland activity in male possums. He concluded that testosterone appeared to be responsible for maintaining scent marking activity in male possums. Castration resulted in the cessation of scent marking activity, although olfactory investigation of conspecific odours did not cease. Scent marking behaviour in castrated individuals implanted with testosterone did not cease. Castration did not, however, appear to change the readiness and ability of an individual to engage in agonistic interactions. This may be related to the animal's previous social experiences; the fact that the castrate was on familiar ground when confronted with an opponent; and/or it may be that the supply of androgens from the adrenal glands that was sufficient to maintain androgen-dependent aggressive behaviours.

Castration resulted in changes in the structural integrity, secretory activity and biochemistry of the scent glands of the possum. The sternal gland appeared to be more responsive to changes in the hormonal state of an individual than the labial or paracloacal glands. Castration resulted in a decrease in the size of sternal gland sebaceous tissue and in the density of the sebaceous glands associated with hair follicles. The relative concentration of the more volatile components of sternal gland secretion was also reduced. Sternal gland structure in individuals implanted with testosterone did not vary from that seen in intact males. The sternal gland secretions of intact and testosterone treated males varied greatly over the 16 months of the study, but neither showed a lack of the more volatile compounds that were lacking the castrated male. The overall size of the paracloacal glands did not change following castration, although there was a reduction in the development of secretory epithelium. As in the sternal gland, the more volatile, low-

boiling point compounds were lost from the secretions of the paracloacal glands. None of these changes were evident in castrates given testosterone. Changes were also seen in labial gland secretions immediately following castration: less volatile compounds and a lower percentage of lipids were found in castrates. As the experiment continued (over 16 months), however, the secretions of the castrate began to more closely resemble that of intact and testosterone treated males. Biggins concluded that testosterone is important for the maintenance of the secretory activity of scent glands and that it possibly influences the odoriferous characteristics of scent gland secretions.

1.3.4. Function of odours

Preliminary observations of the brushtail possum by Jones (1921) suggested that olfaction was not an important sense for this species. He concluded "As is not at all unnatural in an arboreal animal, the sense of smell is by no means highly developed, and it seems to be of little importance in obtaining food or in avoiding enemies". Subsequent studies have shown that olfaction is important in this species. The contexts and possible functions of olfactory communication in the brushtail possum have been studied in some detail, particularly by Winter (1977) and Biggins (1979). Winter made extensive observations of the behaviour (including olfactory communication) of free-ranging possums in a modified area of open forest in Queensland. Biggins conducted a series of experiments investigating the function of odours using captive male possums.

A historical overview of what is known about the context of scent marking behaviours and possible functions of odours, for each cutaneous scent gland, is given below.

Ear, proctodeal, circumanal, interdigital and tail glands
The context of marking and the function of odours associated with these glands are not known.

Chin, labial and salivary glands
The possible functions of odours produced in secretions from chin, labial and salivary glands need to be considered together. This is because the close proximity of these glands, and the high probability that secretions from each may be deposited in the same "chinning" or "labial-sliding" action, make it difficult to attribute any function to a particular gland.

Chinning in males shows a seasonal pattern with a peak in February, and very little between September and January. The main context is the presence of an oestrus female. Winter (1977) suggests the peak corresponds with the early phase of courtship. Further details on the contexts and possible functions of chinning behaviour observed by Winter are given below in the section on chesting.

Biggins (1984) suggests that grooming and washing of the fur using saliva may have a secondary self-anointing function and that information about an individual's identity or physiological state. Saliva may be passively distributed from the syndactylus claws, used in grooming, to the substrate during normal locomotory activities.

Sternal gland
Bolliger and Hardy (1944) proposed that the colour and odour of secretions from the sternal region were probably used to attract the opposite sex. They noted that the colour and odour of the secretion became very noticeable in males at the time of sexual maturity

and that males may mark trees or buildings they inhabit "in order to guide the prospective partner".

Thomson and Pears (1962) suggested that sternal gland secretions were used as territorial markers. Under experimental conditions, however, they reported only a "mild response" by conspecifics to secretions from the sternal gland of other individuals. Kean (1967) observed that the sternal gland was used to mark new objects placed in the pens of captive possums. Possums released from captivity were observed to mark their path at short intervals by pressing their sternal gland against branches and other "prominent features".

As mentioned earlier, males exhibited a seasonal pattern in sternal gland marking with a distinct peak in March and other peaks in June and September. In March and September a higher proportion of marks were made in the context of an oestrus female in the vicinity than in June. Marking in March also had a high proportion of marks made at the marker den. The peaks in March and September correspond with increased sexual activity, with most conceptions occurring at this time. The peak in June corresponded to an increase in marking by two males following the appearance of a young female in their home ranges coming into oestrus for the first time.

Biggins (1979) observed that male possums placed in novel or conspecific odour-marked cages displayed all forms of scent marking, although the frequency of sternal sliding showed the greatest amount of individual variation. Dominant individuals exhibited sternal sliding more often. It was also the most common form of scent marking displayed during agonistic encounters. Biggins suggested that the high level of sternal marking during such encounters is related to the relatively high volatility of sternal secretions compared to secretions from either paracloacal gland. If olfactory communication is important in agonistic encounters the odours deposited would need to be very volatile to be detected by the interacting animals. Secretions from the sternal gland (and other glands) may contain information regarding individual identity. Under experimental conditions conspecifics responded to secretions from scent glands with olfactory investigation and scent marking. The sternal gland and labial glands of conspecifics elicited more marking than paracloacal gland secretions. Paracloacal glands were sniffed but not marked. Biggins demonstrated that males could distinguish between the odours of familiar and unfamiliar males: unfamiliar odours elicited more marking.

Winter (1977) made further observations on chinning and chesting behaviour. Chinning and chesting were the most obvious types of marking observed in the field: in 1160 hours of spotlighting over 4 years Winter observed 333 instances of chinning and chesting (compared to 17 involving urine marking). The majority of these marks were made by males (302/333, ~91%).

Chinning and chesting were performed in a number of contexts. For males, three main contexts for were observed: marking with no other possum in the vicinity (44%), marking with an oestrus female in the vicinity (39%), and marking own den tree (12%). When marking with no other possum in the vicinity, males were observed to make most of their marks while travelling on the ground; other marks were made by males in trees (predominantly chinning), including den trees other than their own. When travelling on the ground the majority of marks were made on tree bases, usually using the sternal gland. Marking occurring with an oestrus female in the vicinity took place either, when the male was following the female on the ground, or when in a tree with the female (either alone or with another male). The majority of these marks are not directed at the female. Marking of den trees sometimes occurred as a male left his den at dusk or as he was returning to a den tree before dawn. Marking was usually more vigorous when returning to a den.

Chinning and chesting in females was less vigorous than in males, usually consisting of a single, light rub. Most marking by females was done in den trees, either when leaving the den at dusk or when returning at dawn. Females did not mark their den as often as males.

Other contexts for marking in females including marking without other possums in the vicinity, marking with a joey following and marking den tree belonging to other individuals. The majority of female marks (24/32, ~77%) were made between the first and last times the joey was seen riding on the mother's back, a time when the female begins to behave more aggressively towards her young. It should be noted that this was only seen in females with female offspring.

Although many marks appear to have been made with no other possum in the vicinity, Winter noted that a high proportion of marking did occur in the vicinity of another possum or in areas known to have been used by other possums. He argues that marking occurs when a possum is aroused by social or other stimuli.

There is no evidence of boundary marking by male possums. Scent marks did not appear to act as deterrent or prevent other males entering the home range of a resident male. Chinning and chesting by males were scattered throughout the home range, but were concentrated in definite areas. The main focal points for marking were dens that were regularly used or visited by the resident male or other males. Other focal points included trees used for feeding.

The response of conspecifics to marks was recorded. In general the response to a chinning or chesting mark made by another individual was minimal, and often marks were completely ignored. The usual response of an adult female to an adult male mark was complete lack of interest — no observations of sniffing or marking were recorded. There is some evidence that adult males will mark over a mark made by another male.

From his observations Winter made a number of suggestions that male possums were able to:

- distinguish between their own odours and those belonging to other males — males sometimes mark where another male has chinned or chested, but do not mark their own marks;
- distinguish between young subordinates and older dominant males — different responses following investigation of tree bases;
- identification of female reproductive state (oestrus and non-oestrus) — investigation of places where females have been sitting, and different responses following investigation of tree bases.

Winter concluded that odours from secretions left by chinning and chesting (and also odours from urine) probably convey messages about the location of an individual and possibly information about the status of the individual. He suggests that the social function of chinning and chesting are to advertise the presence of the marker to a possible rival for a limited resource such as a female or a den.

Pouch

The pouch region of lactating females is very moist with secretions from glandular tissue in the pouch. Biggins (1984) suggests that the secretions may have an odour that is involved in individual recognition between mother and young. When the young first starts leaving the pouch to ride on its mother's back, its fur is stained the same rufous colour as the pouch. The staining diminishes as the young spends less time in the pouch.

Paracloacal glands

Bolliger and Whitten (1948) suggested that the oil-and cell-producing paracloacal glands have different functional roles. This assumption was based on the fact that the secretion of the oil-producing glands is derived from cells which have liquefied and have a distinct odour, whereas the cell-producing gland secretion is composed of intact cells and does not have a discernible odour. They suggested that the oil-producing glands had a role in the defence of the animal and that they may also be involved in "sex behaviour". The only

context noted for release of oil-producing paracloacal secretion was during handling of a struggling animal which resulted in the appearance of the white, oily secretion around the cloaca. No olfactory communication role was suggested for cell-producing gland secretions. Bolliger and Whitten did note, however, that the cells found in the urine remain intact for several weeks (under laboratory conditions). Kean (1967) suggested that although the cells themselves do not have an odour perceptible to humans, their durability may add to the persistence of the odour of deposited urine. It is also interesting to note that Bolliger and Whitten found that the cells started appearing in the urine of juvenile animals as soon as they started leaving the pouch. Juveniles of both sexes were found to generally have exceptionally large numbers of cells in their urine. In adults, male urine contained more cells than female urine, which sometimes had very few cells in it.

Thomson and Pears (1962) suggested that secretions from the paracloacal glands are important in recognition of sex and serve as a territorial marker in the possum. A series of experiments using pieces of tissue soaked with paracloacal secretions were conducted. Secretions from the oil-secreting paracloacal glands of females elicited a marked sniffing response in males. Secretions from males caused other males to adopt a threat posture, in which they were observed to sniff loudly, rise onto their hind legs, retract the scrotum and lean forward over the source of the odour with out-stretched limbs, often thumping their tail and repeatedly making a sharp "T'cher" vocalisations. Some females also responded in a similar manner to male oil-secreting paracloacal gland secretion. No definite response was observed in experiments using secretions from the cell-producing paracloacal glands. Reasons for a possible territorial function for paracloacal gland secretions were not elaborated upon in this paper.

Kean (1967) observed, in captive conditions, that the paracloacal glands were not used often. Evacuation of secretions from the oil-producing glands was observed in frightened animals. Kean proposed that urine, which contained paracloacal gland secretions, deposited during urine-dripping behaviour was associated with "proprietary rights". Such marks were said to be recognised by the marker whose behaviour was described as persistent. Recognition of the markers ownership by other possums was shown by avoidance, although "some variation" between individuals was seen. Relocation of captive animals to a new pen was observed to induce urine-dribbling behaviour. Kean suggests that it is difficult to defend a three-dimensional territorial space and that use of urine-dribbling to mark parts of a territory may have a repellent effect on other individuals, thus reducing "territorial claims".

As mentioned earlier urine-dribbling, cloacal dragging and paracloacal gland evacuation are rare events in free-ranging possums (Winter 1977). Active marking with the white oily paracloacal secretion was never seen in the field. Urine marking was performed by males and females, although only 17 instance of urine-dribbling were recorded in 1160 hours of spotlighting over four years. Most of the male observations occurred when the male was in a tree with an oestrus female. All the female observations occurred at the time when the mother-joey bond was beginning to break down. The only response to a urine mark from another individual was a brief sniff. Winter suggested that if cells from the cell-secreting paracloacal glands do contribute to the odour of urine, then information about age and sex are probably contained in urine marks.

Urine dribbling and cloacal dragging were recorded in encounters between a male and female possum in captivity (Wemmer & Collins 1978). These behaviours followed, or alternated with, chinning behaviour in the male. No mention of a possible function is made.

Biggins (1979) observed that captive possums saturate their cage with secretion from sternal and paracloacal glands. He suggested that paracloacal secretions, which contain less volatile compounds than sternal secretions, may be primarily related to demarcation and recognition of familiar home grounds. Marking using paracloacal gland secretions was seen more often in subordinate male possums. The difference in marking behaviour

between subordinate and dominant individuals may enable possums to distinguish between conspecifics of different status. In vigorous attacks, adoption of submissive postures and evacuation of the oil-producing paracloacal glands by a subordinated individual appeared to appease the attacker. This observation highlights one of the difficulties in determining the function of odours in animal behaviour. Other communicatory behaviours, particularly visual or auditory ones, may be associated with olfactory cues making it difficult to determine the functional role of each.

Although the secretions of the two types of paracloacal glands are structurally different (whole-cell verses liquefied cells) (Bolliger & Whitten 1948), Biggins (1979) and Allen (pers. comm cited in Russell 1985) have demonstrated that the chemical composition of the secretions are very similar. Furthermore, the most common method of distribution of oil- and cell-producing paracloacal secretions is probably by passive deposition with urine and faeces during normal eliminatory activities (Bolliger & Whitten 1948; Biggins 1979). Russell (1985) proposes that the combined secretion has a short-term, immediate effect which comes from the odorous oil-gland secretion, and a longer term component that is released from the cell-gland secretion as cells begin to break down. The view of Thomson and Pears (1962) and Kean (1967) that paracloacal secretions are the main territorial marker is not held by Russell.

As discussed earlier, possums do not appear to use odour from any source as a territorial boundary marker and odour marks do not appear to prevent conspecifics from entering the home range of others. Biggins (1979) has proposed that scent marking (using secretions from sternal, labial and paracloacal glands) serves the saturate the home range with familiar odours with increase the self-confidence of the resident. In a series of experiments resident males were consistently successful in their encounters with intruders. Indeed, in some cases it was possible to reverse the outcome of an agonistic encounter between two males by reversing the level of familiarity of the surroundings.
Rather than acting as a threat and deterring a potential intruder, scent marking and the establishment of a familiar home ground may serve in the formation an area in which the resident male is dominant over a male intruder.

A summary of the range of possible communicatory functions of odours and scent marking derived from the studies conducted by Winter (1977) and Biggins (1979) is shown in Table 2. The table is adapted from reviews by Biggins (1984) and Russell (1984).

Table 2. Proposed communicative function of scent-marking in the brushtail possum *Trichosurus vulpecula.*
(adapted from Biggins (1984) and Russell (1984); based on Winter (1977) and Biggins (1979)).

Odour Source	Mode of scent deposition	Object marked	Signalling individual	Information conveyed/ Possible function	Response of receiver
mouth (chin & labial glands; saliva)	chinning chewing labial sliding	substrate — ground, trees (especially braches) conspecific odour marks	♂ > ♀	individual identity social status sex age home range familiarisation	approach or withdrawal, and over-marking — depending upon s over-marking by chinning and/or chewing
	self-grooming	self (substrate passively)	♂, ♀	as above	not specified
sternal glands	sternal-sliding/chesting	substrate — ground, trees (especially bases) conspecific odour marks	♂ > ♀	as above	approach or withdrawal, and over-marking — depending upon s over-marking by chewing, chinning, and/or chesting
paracloacal glands (cell secreting)	urination, defecation (passive emission)	substrate	♂, ♀	as above (long term signal)	not specified
	urine dripping	substrate	♂, ♀	as above (long term signal)	approach/avoidance, depending upon status
paracloacal glands (oil secreting)	urination, defecation (passive emission)	substrate	♂, ♀	as above	as above
	cloacal-drag	substrate	♂, ♀	as above	as above
	copious evacuation	self (when loser in an agonistic encounter)	♂, ♀	fear, submission	inhibition of aggression
other paracloacal glands	passive emission	substrate, self	♀	sex, reproductive status	♂ approach if ♀ in oestrus
pouch glands	passive transfer	self, pouch young	♀	sex, reproductive status, location of pouch and identity of mother and young	♂ approach if ♀ in oestrus bond between mother and young

1.4. Aim of study

As the preceding review has shown olfactory communication has been studied in some detail in the brushtail possum. Location and structure of scent glands, the chemical composition of secretions, the role of hormones, the range of scent marking behaviours, the context of marking behaviours, the response of conspecifics to odours, and the possible function of odours have all been investigated. Information has been collected from a variety of observational and experimental studies using captive and free-ranging animals. Furthermore, data on olfactory communication has been integrated with the extensive knowledge of the biology, ecology and social organisation of the species.

Despite the broad range of information already collected, there are a number of aspects of olfactory communication in the brushtail possum that have not been explored in any detail, and there are many questions about the function of odours that remain unanswered.
The broad aim of this study is to continue the investigation of olfactory communication in the brushtail possum by focusing on the sternal gland. Three main areas will be explored.

The first is an examination of the histology and gross morphology of the sternal gland. To date no detailed investigation of any differences or changes the structure of the sternal gland in relation to gender, age, status, or season has been conducted. Samples of sternal gland tissue from roadkill possums collected over a 12 month period were examined.

The second focus is the development of a suitable method of recording scent marking under natural conditions; one that can be easily adapted for use in other species that are nocturnal, arboreal and cryptic. Although Winter (1977) was able to observe olfactory communication in the brushtail possum under natural conditions using spotlighting, it is generally found that such observations, even in the most open habitats, are difficult to do without disturbing or altering the normal behaviour of the animals. Indeed, Winter reported that using a spotlight did effect the behaviour of some individuals. Unfortunately, most studies do not have the resources to make observations over extended periods of time: Winter spent 1160 hours over 4 years spotlighting possums. The aim of this part of the study is to develop a cheap, reliable, easy to use method for obtaining information about scent marking under natural conditions.

The third purpose of this study is to record the use of the sternal gland in the brushtail possum under natural conditions with the aim of better understanding its function. This gland is intrinsically interesting because it occurs in both males and females, but shows a degree of sexual dimorphism in its appearance, the timing and proportion of use, and the chemical composition of its secretions.

Chapter 2. Histology of the Sternal Integument

2.1. Introduction

The sternal integument of the brushtail possum was first described in detail by Bolliger and Hardy in 1944. Externally, the sternal integument, or "sternal gland", is visible as a sparsely haired oval, triangular or diamond shaped area of stained fur overlying the sternum (Bolliger 1944a & b; Bolliger & Hardy 1944; Green 1963). The area is easily distinguished from the surrounding integument in that the hairs in mature animals are a red-brown colour due to the presence of pigment in the medulla and cortex. Staining of the fur with secretions from the underlying glandular tissues further enhances the distinctive appearance.

Secretions from the sternal gland are deposited on the substrate in a forward rubbing movement variously known as "sternal marking", "chesting", "sternal sliding" or "sternal rubbing". The possum adopts a bent leg stance and lifts its chin up enabling the chest to come in contact with the substrate. In a forward motion the chest is rubbed up the substrate and then lifted off. The action may be repeated a number of times. Tree trunks, branches, fallen logs, bare earth and tufts of grass are marked in this way (Winter 1977).

The sternal gland occurs in both males and females. An examination of pouch young of various ages by Jones (1920) did not reveal "any naked-eye trace of the 'chest gland' said to characterise the adult". Bolliger and Hardy (1944), however, observed that during the third month of pouch life a narrow band of grey pigmentation appears over the sternum, followed by the appearance of grey or black hairs. At approximately three months of age the pigmented sternal strip is the same width in males and females. The length in females is approximately 25mm, whereas in males it extends further up the neck and is about 35mm in length. At 7-8 months the hair of the sternal region is coarser in texture and darker in colour than the surrounding fur. Between 8 and 12 months the whole length of the sternal hair shaft takes on a brown colour. This process occurs more rapidly and is more pronounced in males than females. In comparison with hair on other parts of the body, hair on the sternal region is shorter (average length of 5-10mm), thicker (32μm) and more sparse.

As possums become sexually mature the area of brown fur on the sternum becomes increasing obvious. In sexually mature animals there are gender differences in the external appearance of the sternal integument. In males, it is characteristically triangular or diamond shaped due to lateral extension at the level of the forelegs and extension along the neck, anterior to the sternum. The size in males may be up to 8-10cm long and 3-5 cm wide. Secretions from the gland make the fur oily, particularly during the breeding season. In females development is not to the same degree as in males. The sternal region is usually an elongated oval shape between 5-7cm long and 1-3cm wide that extends anteriorly in a narrow band as far as the end of the sternum. The fur appears almost dry in the breeding season, but becomes moist 2-3 months after the young is born (Bolliger and Hardy 1944).

Histologically, the sternal integument is composed of holocrine sebaceous and sudoriferous apocrine glands that are much larger and more active than those on the general body surface. According to Bolliger and Hardy (1944) the position and arrangement of these

and other structures in the sternal integument are essentially similar to those found in other parts of the integument. There are, however, both absolute and relative differences in the sizes of certain structures and the apparent degree of activity of the glands. A list of the major histological differences between sternal skin and skin else where on the body is given below:

- the sternal skin is 50-100% thicker — due to a slightly thicker stratum malpighi and a much thicker dermis;
- the hair follicles of the sternal skin have fewer lateral clusters for each central follicle and the number of fibres in each lateral cluster is lower;
- the number of follicles (both central and lateral) per area of integument is lower in the sternal region;
- the follicles are more deeply implanted in the sternal integument, and the average fibre thickness is greater; and
- both the holocrine sebaceous and sudoriferous apocrine glands are larger and apparently more active in the sternal region.

A series of experiments involving castration and administration of exogenous hormones have demonstrated that the development and maintenance of the sternal integument is primarily influence by testosterone (Bolliger 1944a & b; Biggins 1979).

Although the structure of the sternal integument has been described there has not been a detailed study of age, gender, social status, reproductive state or seasonal differences in the histology of the gland. Biggins (1979) collected samples of sternal skin, and labial and paracloacal glands from five captive individuals (4 adult males — 3 "normal" and one socially-stressed; one adult female, one subadult (14 months) male, and; one juvenile (9 months) male). For the sternal tissue, he reported that the sebaceous glands of the adult males were "large and densely clustered around the sternal hair follicles" indicating they were very active. Conversely, the sebaceous glands of the adult female, the subadult and juvenile males, were small and occurred in smaller clusters than in the adult males. In the socially stressed male the sebaceous elements were markedly atrophied and there were no large clusters around the hair follicles. The sudoriferous apocrine tissue was not discussed.

This purpose of this chapter is not to redescribe the histology of the sternal integument, but to compare and contrast differences in the histology of the gland between and within the sexes, between mature and immature animals, and between groups of possums over different seasons. No detailed accounts of seasonal changes in the histology of scent glands in mammals have been reported in the literature to date. This study aims to address the lack of seasonal information on the histology of mammalian scent glands. Examination of gender, maturity and seasonal differences in the histology of the sternal gland will enhance understanding of differences in the scent marking behaviour of individual brushtail possums, which is discussed in Chapter 5.

2.2. Methods

2.2.1. Collection and Preparation of Samples

Roadkill brushtail possums (119 males and 52 females) were collected from Tasmanian roads between July 1991 and June 1992 (Department of Parks, Wildlife and Heritage Permit no. 138/91). Tasmanian roads provide an abundant and hitherto under-exploited source of animals, particularly brushtail possums, for research purposes. Possums were collected from most areas of the state, although the majority came from the north-west, Midlands and southern regions, with some individuals from the west and east coasts and Central Plateau. Collection occurred from sea-level to an elevation of approximately 1000m. A record was made of the location and condition (ie missing external or internal body parts, relative "freshness") of the roadkill. Animals were either examined immediately or frozen — only animals that appeared to have all body parts present and had no evidence of tissue decomposition were used.

Measurements of head, ear, manus, pes, and tail length were made according to the methods of Lyne & Verhagen (1957). (It should be noted that many animals with extensive damage to the head region were included in the study even though it was not possible to measure the size of the head. Exclusion of these animals would have resulted in a small study sample.)

For female possums the presence or absence of a pouch, the condition of the pouch, and the presence or absence, and size of pouch young were recorded. For males the length, breadth, width and weight of the testes was recorded. The length and breadth of sternal staining was recorded for both sexes.

A sample (approximately 15 x 15 mm) of the sternal integument (see Plate 1) overlaying the centre of the clavicles was excised from each roadkill. The samples were immediately placed in Bouin's fixative. A small (approx 5 x 10mm) section of each tissue sample was passed though a series of dehydration alcohols, cleared in Histo-A and embedded in paraffin wax under vacuum. Transverse sections of 7 to 8μm were cut using a rotary microtome, stained using Mallory's triple stain and mounted in Depex.

2.2.2. Measurement of Samples

The following tissue parameters were measured (see Plates 2-4):

1. total depth of the glandular tissue from the top of the epidermis to the base of the sudoriferous apocrine tissue.
2. thickness of the epidermis
3. depth of the holocrine sebaceous tissue
4. depth of the sudoriferous apocrine tissue
5. diameter of holocrine sebaceous and sudoriferous apocrine cell nuclei
6. the cell height and lumen diameter of the sudoriferous apocrine tissue
7. the percentage of holocrine sebaceous tissue
8. the percentage of sudoriferous apocrine tissue

Parameters 1 to 6 were measured using a conventional microscope-graticule setup. For each animal, ten measurements of each parameter were made — each one from a different slice of the sectioned material. Each parameter was measured to the nearest 0.1μm (after calibration).

Measurement of the percentage of each glandular tissue type was facilitated using the image-processing software *Mocha* (Jandel Scientific). Digital images of each section were captured using the *Mocha* via a video-camera mounted on the microscope. The area of each glandular tissue type and the total amount of tissue were assessed by measuring around the outer edge of each using the image processing software tools. This enabled the percentage of each tissue type to be calculated. For each animal one section of tissue was measured.

2.2.3. Grouping of animals and data for analysis

2.2.3.1. Male maturity and reproductive state

The literature provides a number of ways of distinguishing between juvenile and adult males. The published data are based on small sample sizes and do not necessarily relate well to the sample of animals in this study. Gilmore (1969) and Smith *et al* (1969) observed that spermatozoa were absent from the testes of most possums with a testis weight <2 g. Tyndale-Biscoe (1955) showed a correlation between the presence of spermatozoa and the length of the testes. Spermatozoa were not found in males with a testis length ≤ 17mm.

In this study juvenile and adult male possums were distinguished by weight. Males possums less than or equal to 2 kg body weight were considered to be juveniles; individuals with a body weight greater than 2 kg was considered to be adult. This cut off point was made on the basis of the following observations.

Plotting testis weight against body weight for the males collected in this study shows a clear separation in the testis weight at a body weight of 2 kg (see Figure 1). At body weight greater than 2 kg there was an obvious increase in to the weight of the testes. This is consistent with the observation of Gilmore (1969) that although there is little increase in testis size relative to body weight in immature animals, at puberty the testes increase in size very rapidly. This occurred at a body weight of approximately 2.5kg in Gilmore's study. Similar results were reported by Smith *et al* (1969). Testis weight was observed to increase little until a body weight of 1.5kg was reached. Following the onset of spermatogenesis weight increased sharply. Males with body weights of 1.5kg or greater were considered to be mature.

There is no seasonal variation in testis size and spermatogenesis in mature males (Bolliger & Carrodus 1938a; Bolliger 1942, 1946; Tyndale-Biscoe 1955; Dunnet 1956; Gilmore 1969). The prostate, however, shows marked seasonal variation, with the greatest mean weights being recorded during the two times of the year when females are in oestrus (Gilmore 1969). No measurements of the prostate were made in this study.

Plate 1. Sternal integument of a male roadkill possum showing the sparse covering of hair overlying the sternum and the discolouration of the surrounding hair from secretions produced by the glandular tissues.
Scale: black square = 1 x 1cm
(Photo: R. Mawbey)

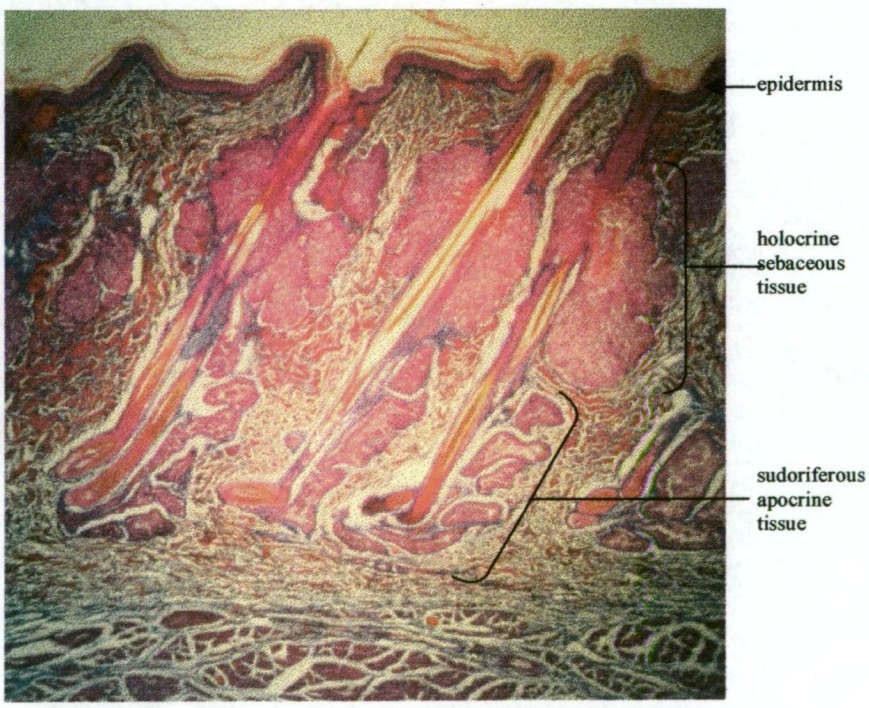

Plate 2. Transverse section of the sternal integument of a male brushtail possum showing the holocrine sebaceous and sudoriferous apocrine tissues.
Scale: 0.5mm (Photo: K. Hynes)

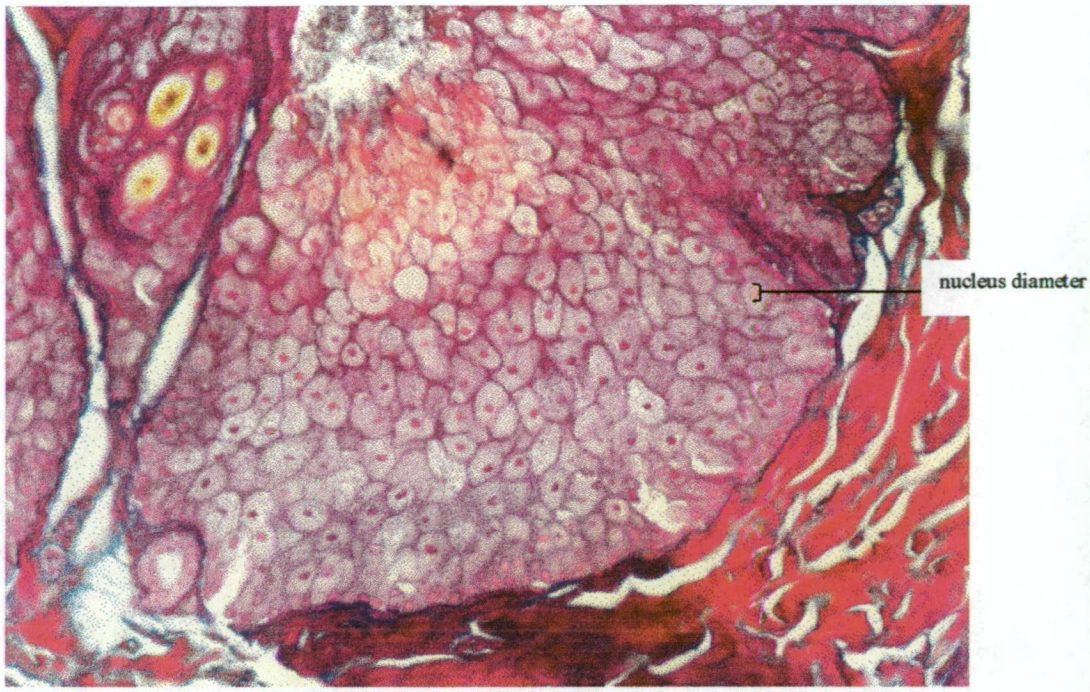

Plate 3. Transverse section of the sternal integument o f a male brushtail possum showing holocrine sebaceous nuclei.
Scale: ⌊_____⌋ (Photo: K. Hynes)
100μm

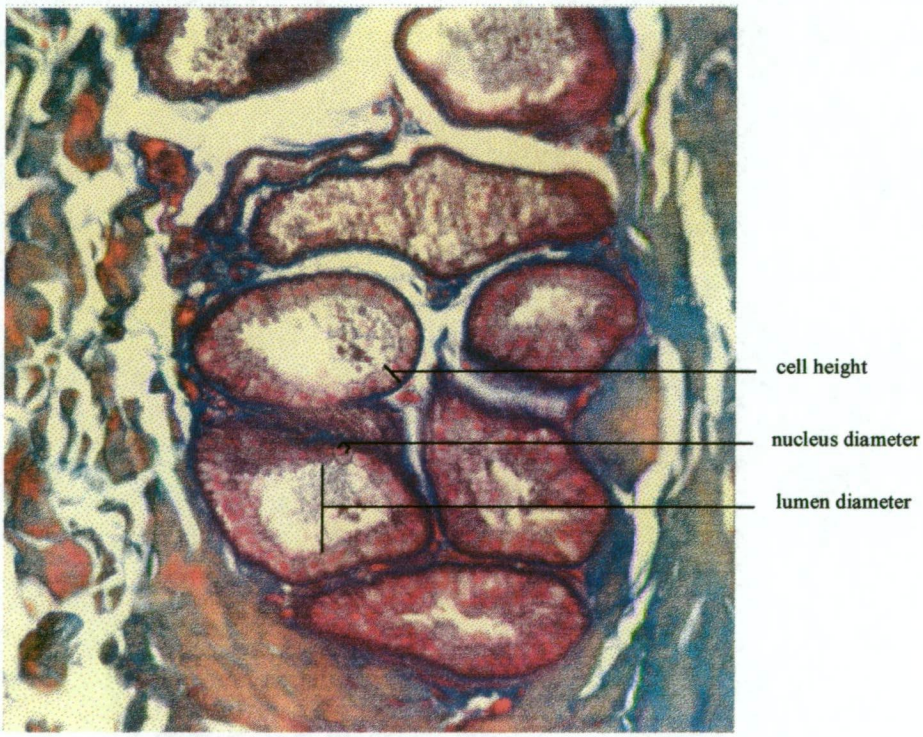

Plate 4. Transverse section of the sternal integument of a male brushtail possum showing the sudoriferous apocrine cells, nuclei and lumen.
Scale: ⌊_____⌋ (Photo: K. Hynes)
75 μm

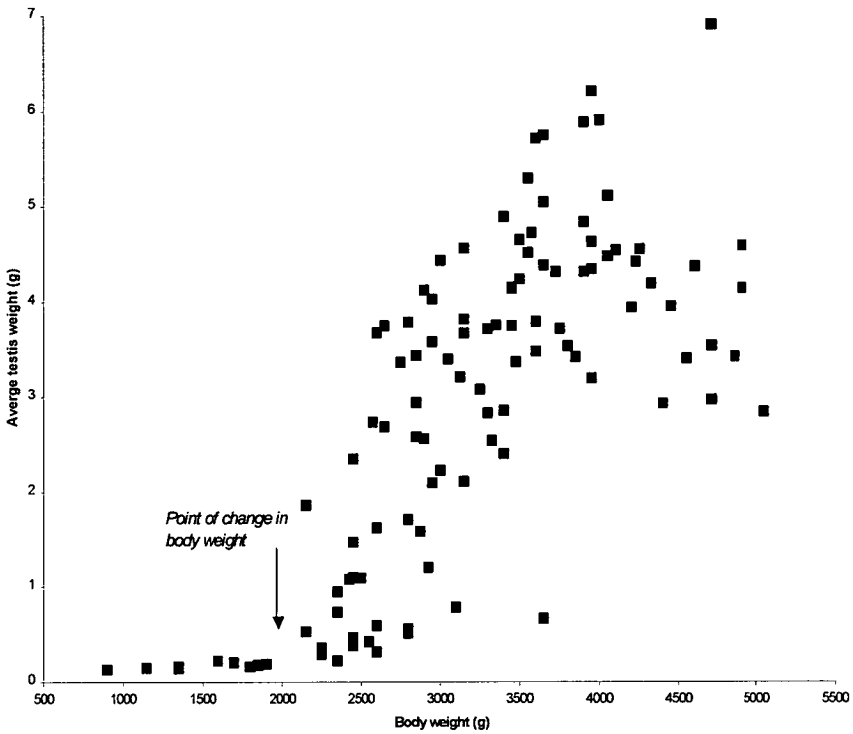

Figure 1. Relationship between body weight and testis weight in male roadkill possums.

2.2.3.2. Female maturity and reproductive state

Maturity of female possums can be determined by examination of the pouch and nipples. In immature females the pouch is unformed and the nipples are inverted. Mature females have fully formed invaginated pouches and everted nipples (Hocking 1981). Using the condition of the pouch and nipples Hocking (1981) further subdivided mature females into 4 groups as a way of determining their recent breeding history:

- Mature animals in oestrus condition (ie pouch clean and moist with a waxy secretion and a slight reddening around the nipples).
- Mature animals carrying pouch young.
- Mature animals without pouch young but lactating.
- Mature animals in anoestrus (ie pouch noticeably drier).

(Note: Hocking based his classification of mature females on the work of Sharman (1962)).

Unfortunately, due to the nature of the roadkill sample it was not possible to use same information as Hocking and Sharman to classify females by their reproductive state. Immature females and those carrying pouch young could be easily distinguished. Those without pouch young were more difficult to classify. It could not be determined conclusively whether lactation related to a pouch young that had evacuated the pouch following the mother's death, or whether it was associated with a dependent young that had not been weaned. Lactating females were therefore grouped together, even though it would have be preferable to separate them, as Hocking and Sharman did, into two groups based on the developmental stage of the young. For the purposes of this study females were placed into one of four groups based on the following observations:

1. Immature females — Pouch not formed.
2. Mature female in oestrus — Pouch formed, but not containing dry, crusty brown-coloured secretion; nipples showing little or no elongation; mammary glands not swollen.
3. Mature females with pouch young or with dependent, unweaned young — Pouch formed with pouch young present, or pouch formed and evidence of a pouch young or dependant on back or at heal. Evidence based on the lactating status of the females, ie one nipple elongated; one mammary gland swollen; and milk able to be expressed by squeezing the mammary gland.
4. Mature females in anoestrus — Pouch formed, and containing dry, crusty brown-coloured secretion; nipples showing little or no elongation; mammary glands not swollen.

2.2.3.3. Seasonality

Possums have a distinct breeding cycle, although births have been recorded in all months of the year. The majority of births are in autumn between April and June although a secondary spring breeding season in September occurs in some locations (Bolliger 1940; Tyndale-Biscoe 1955; Dunnet 1956, 1964; Gilmore 1969; Crawley 1973). A single young is usually born and it spends the next 4-5 months attached to one of two teats in its mother's pouch were its develops rapidly. A further 1-2 months are spent suckling and riding on the mother's back before weaning occurs (Dunnet 1956, 1964; How 1983). The age of independence and subsequent dispersal appears to be highly variable (see Dunnet (1956), How (1972) and Hocking (1981)), although approximately six months of age is usual. As most young are born between April and June there is a period between October and December when young animals are dispersing.

For the purposes of this study the year has been divided into four equal time periods of three months each:

- *January-February-March — Pre-Breeding Season*
  Female in anoestrus moving into oestrus. Some births in March.
- *April-May-June — Breeding Season*
  Females in oestrus. Most births occur.
- *July-August-September — Post-Breeding Season and Secondary Breeding Season*
  Females have young in the pouch. Females without pouch young come into oestrus and may breed (in September).
- *October-November-December — Dispersal Period*
  Dispersal of young from autumn breeding season.

2.2.4. Statisitical analysis

Discriminant analysis (also known as canonical variate analysis) techniques (Phillips *et al* 1973) were used to look for differences across gender, maturity and seasonal groups in terms of the histological parameters. The statistical software SAS was used.

The histological parameters were checked for the assumption of normality. Three parameters required transformation:

Tissue Parameter	Transformation
• holocrine sebaceous tissue depth	square root
• sudoriferous apocrine nuclear diameter	natural logarithm
• sudoriferous apocrine cell height	natural logarithm

For each combination of gender, maturity and season to be compared a series of steps were taken in the analysis. Univariate tests were used to examine the importance of each histological parameter in separating the groups being compared. Pair wise comparisons of the groups are shown for those histological parameters that were able to significantly separate the groups. The mean values and standard errors of the parameters are also presented. Stepwise canonical variate analysis of all the histological parameters was performed to determine which of the histological variable(s) gives the greatest group separation.

For some of the groups further analysis was performed. The details are given below with the relevant groups.

Note:

1. Male possum M4 was not included in any of the analyses as this animal had very strange holocrine sebaceous nuclei (most were very small and "withered", while a few were more then 3x the size of those found in other animals). Its inclusion in the analyses resulted in skewing of the results. It was a very distinct outlier.
2. There are 15 animals missing from the analyses of the following sudoriferous apocrine tissue parameters: nuclear diameter, cell height and lumen diameter. Thirteen of the animals (males M40, M44, M62, M64, M66, M70, M84, and M102, and females F6, F9, F24, F28, and F48) were excluded because the sudoriferous apocrine tissue components were not clear enough to be measured accurately. The remaining two animals, males M57 and M116, were unusual in that their sternal gland tissue contained extremely small amount of apocrine tissue; indeed for most sections no apocrine tissue could be discerned. When included in the analysis both these animals were outliers and had some influence on the results. These males were also excluded from the analysis of sudoriferous apocrine tissue depth, and M116 was excluded from the analysis of percentage sudoriferous apocrine tissue. M57 was included in the percent tissue analysis as the section chosen at random for measurement did contain some sudoriferous apocrine tissue.

2.2.4.1. Gender and maturity

Three sets of analysis were performed using gender and maturity.

Firstly, four gender-maturity groups were compared (ie immature males, immature females, mature males and mature females); two male-maturity groups were compared (ie immature males and mature males), and two female-maturity groups were compared: (females without a pouch, ie the immature, and females with a pouch, ie the mature). The initial analyses (as outlined above) were performed on each of the three gender-maturity sets.

Following the initial analysis some further tests were performed on the first gender-maturity set. Cross-validation using a linear discriminant function was used to look at the level of error associated with the classification of the gender-maturity groups.

Factor analysis was used to identify relationship between the histological parameters. A multivariate analysis of variance was performed using the three distinct gender-maturity groups identified in the discriminant analysis above (ie. immature individuals, mature females and mature males) in order to generate residuals on which the factor analysis could be performed. Residuals were used to eliminate any influence the gender-maturity groups may have had on the factor analysis. Results for the unrotated factor pattern and the rotated factor pattern (varimax) are given.

The common factors were then used to look at differences between the gender-maturity groups. The standardised scoring coefficients of the factor analysis were applied to the histological parameters (rather than the residuals) to create values for the four factors for each individual. A stepwise procedure was used to identify which factor(s) were important in separating the gender-maturity groups. Cross validation using a linear discriminant function was then applied to look at the level of errors associated with group classification based on the identified factors.

2.2.4.2. Gender by season

Three sets of analysis were performed using gender and season. Only mature individuals were compared in these analyses. In the first, eight gender-season groups were compared, ie males and females collected in each of the four seasonal time periods (pre-breeding, breeding, post-breeding/secondary breeding and dispersion). The four seasons were then compared using the sexes separately.

2.2.4.3. Females by reproductive status

Two sets of analyses were performed comparing females of different reproductive status. In the first four groups were compared: immature females, anoestrus females, oestrus females, and females with young. In the second analysis only the three mature female groups were compared, ie the immature individuals were removed.

Table 3 outlines the analysis strategy.

Table 3. Strategy for analysis of gender, maturity, seasonal and reproductive differences in the histology of the sternal gland of the brushtail possum.

Analysis	Gender	Level of maturity	Seasons	Reproductive state
1	♂ and ♀	immature and mature		
2	♂	immature and mature		
3	♂	mature only	pre-breeding breeding post-breeding dispersal	
4	♀	immature and mature		
5	♀	mature only	pre-breeding breeding post-breeding dispersal	
6	♀	immature and mature		immature anoestrus oestrus pouch young
7	♀	mature only		anoestrus oestrus pouch young

2.3. Results

2.3.1. Gender and maturity

2.3.1.1. Males and females by maturity

Table 4 shows the results of the univariate analysis of the individual histological parameters. The following tissue parameters (shaded on table) showed significant differences between the gender-maturity groups: total tissue depth, holocrine sebaceous depth, sudoriferous apocrine depth, sudoriferous apocrine lumen diameter, percentage holocrine sebaceous tissue and percentage sudoriferous apocrine tissue. The gender-maturity groups could not be significantly separated by the size of the cell nuclei for either tissue type, or by the height of the sudoriferous apocrine cells.

Table 4. Univariate test for gender-maturity group separation.

Tissue Parameter	Transformation	F ratio	P
Total tissue depth	none	F(3, 166)=24.5437	P=0.0001
HS tissue depth	square root	F(3, 166)=25.3811	P=0.0001
SA tissue depth	none	F(3,164)=15.1300	P=0.0001
HS nuclear diameter	none	F(3, 166)=0.1893	P=0.9035
SA nuclear diameter	natural logarithm	F(3,151)=0.3026	P=0.8235
SA cell height	natural logarithm	F(3, 151)=1.4858	P=0.2207
SA lumen diameter	none	F(3, 151)=5.9011	P=0.0008
Percent HS tissue	none	F(3, 166)=6.7370	P=0.0003
Percent SA tissue	none	F(3, 165)=6.2360	P=0.0005

Key: HS holocrine sebaceous
SA sudoriferous apocrine

Pair-wise comparisons of the tissue parameters shown to be significant in the univariate analysis are shown below in Table 5. There were no differences between immature males and females for any of the tissue parameters. The mean values for each of the tissue parameters for each of the groups is shown in Table 6. Because there are no significant difference between male and female immature animals the mean values for all immature animals is shown.

For all the significant comparisons the values of each tissue parameter all show the same trend. That is, immature animals have the smallest values, followed by mature female animals, with mature males having the largest mean tissue value.

Table 5. Pair-wise squared differences between gender-maturity groups from single variable stepwise discriminant (canonical variate) analysis.

Tissue Parameter	Immature ♀: Immature ♂	Immature ♀: Mature♀	Immature ♀: Mature ♂	Immature ♂: Mature ♀	Immature ♂: Mature ♂	Mature ♀: Mature ♂
Total tissue depth	P=0.1598	P=0.0045	P=0.0001	P=0.0001	P=0.0003	P=0.0001
HS tissue depth (square root transformed)	P=0.6594	P=0.0012	P=0.0001	P=0.0016	P=0.0001	P=0.0001
SA tissue depth	P=0.8906	P=0.0019	P=0.0001	P=0.0202	P=0.0001	P=0.0071
SA lumen diameter	P=0.8881	P=0.0100	P=0.0008	P=0.0279	P=0.0067	P=0.5128
Percent HS tissue	P=0.5818	P=0.3046	P=0.0007	P=0.8140	P=0.0399	P=0.0024
Percent SA tissue	P=0.6028	P=0.1433	P=0.0005	P=0.5277	P=0.0311	P=0.0124

Significant differences (P≤0.05) are shaded

Table 6. Tissue parameter means (μm) and standard errors.

Tissue Parameter	Immature ♂ &♀	Mature♀	Mature ♂
Total tissue depth	1045.6 (± 65.5) (♂: 907.9 ± 118.6) (♀: 1126.77 ± 72.8)	1457.6 (± 51.3)	1734.9 (± 40.8)
HS tissue depth (square root transformed)	17.0 (± 0.6) (♂: 16.6 ± 1.1) (♀: 17.3 ± 0.7)	21.6 (± 0.6)	24.9 (± 0.5)
HS tissue depth (reverse transformed)	299.9 (♂: 285.7) (♀: 308.9)	479.9	643.2
SA tissue depth	345.2 (± 33.1) (♂: 352.6 ± 73.6) (♀: 341.3 ± 34.3)	526.8 (± 33.2)	634.6 (± 19.8)
SA lumen diameter	21.8 (± 2.3) (♂: 21.2 ± 3.8) (♀: 22.2 ± 2.8)	35.5 (± 3.7)	37.5 (±1.6)
Percent HS tissue	10.3 (± 0.9) (♂: 11.1 ± 1.5) (♀: 9.9 ± 1.2)	11.5 (± 0.8)	14.7 (± 0.5)
Percent SA tissue	4.9 (± 0.7) (♂: 5.3 ± 1.3) (♀: 4.6 ± 0.9)	6.2 (± 0.6)	7.9 (± 0.3)

Differences between means in the shaded boxes are **not** significant (P>0.05).
There are no significant differences (P≤0.05) between immature males and immature females.

Stepwise canonical variate analysis showed that only one histological parameter is required to maximally separate the gender-maturity groups. The transformed (square root) sebaceous depth parameter ($F_{(3, 166)}$=25.3811, P<0.0001) on its own provides enough information to enable separation of the four gender-maturity groups. (Note: Total tissue depth ($F_{(3, 166)}$=24.5437, P<0.0001) is almost as good as holocrine sebaceous tissue depth in separating the gender-maturity groups. This is to be expected as the holocrine sebaceous tissue composes most of the total tissue depth). The result of requiring only one tissue parameter to maximally separate the gender-maturity groups is that the p-values for differences between the gender-maturity groups are the same as those shown in Table 3 for holocrine sebaceous tissue depth.

There was no significant difference between the sexes in immature animals. Mature males and females differed from each other and from immature animals significantly.

Table 7. Percent classified into each group.

Group	Immature ♀	Immature ♂	Mature♀	Mature ♂	Total n
from Group					
Immature ♀	35.29 (n=6)	41.18 (n=7)	23.53 (n=4)	0.0 (n=0)	17
Immature ♂	40.00 (n=4)	50.00 (n=5)	10.00 (n=1)	0.00 (n=0)	10
Mature♀	11.43 (n=4)	14.29 (n=5)	31.43 (n=11)	42.86 (n=15)	35
Mature ♂	7.41 (n=8)	4.63 (n=5)	19.44 (n=21)	68.52 (n=74)	108
	22	22	37	89	170

Table 7 shows the results of the cross-validation analysis. Some immature males were classified as immature females and *vice versa.* This is not unexpected given that the stepwise discriminant analysis showed no significant difference between immature males and females. The majority (~82%) of immature animals (regardless of sex) were classified as being immature. Almost one quarter of the immature females, however, were classified as mature females; none were classified as being mature males. All four of the immature females classified as mature females were collected between January and March in the pre-breeding season. These four females weighed on average more (2575.0 ± 278.4g) than correctly classified immature females (2167.3 ± 379.9g) (t-test: p=0.068). One immature male was classified as a mature female. This individual was collected in October, had a body weight of 1700g and an average testis weight of 0.2g. All the sternal integument tissue parameters (except the sudoriferous apocrine lumen diameter) shown to be significant in the univariate analysis above were much larger than in other males of comparable weight (see Table 4): total tissue depth = 1384.9 μm; holocrine sebaceous depth = 491.4 μm; sudoriferous apocrine depth = 629.4 μm; sudoriferous apocrine lumen diameter = 14.1 μm; percent holocrine sebaceous tissue = 19.3%, and percent sudoriferous apocrine tissue = 13.4%. The area of staining on the sternum (6.0 x 0.5cm) was not any greater than in other immature males.

Among the mature females there was much misclassification. Only 32% of mature females were classified as such. All of these females, except one, were collected in May, June, July and August, and the majority (8/11) had pouch young or evidence of a pouch young or a young not yet weaned. The remaining three showed evidence of oestrus. It is

possible, however, that two of these females were incorrectly classified: both were collected in July and may possibly have had pouch young that had evacuated the pouch following the mothers' death. The remaining female was collected in November. This individual weighed less than all other females in this group and not much more than an average immature female (2550g compared to average immature weight of 2263.2 ± 393.1g). It is probable that this female was oestrus cycling for the first time.

Approximately 26% of mature females were classed as being immature animals. These females were collected in most months of the year and in all three reproductive states. On average they weighed less (2966.7 ± 314.3g) than mature females classified as mature (3329.6 ± 681.5), although this is not a significant difference (t-test: p=0.159).

The majority of mature females (43%) were classified as being mature males. Two-thirds of these females were collected during the pre-breeding and breeding season, although individuals were collected in most months of the year. Over half had pouch young or evidence of a pouch young or a young not yet weaned, a third were in anoestrus, and the remainder in oestrus. The average weight of mature females classified as mature males was greater (3631.7 ± 432.2) than mature females classified as mature females, but the difference is not significant (t-test: p=0.179).

The majority of mature males (~69%) were classified correctly. Approximately 19% were classified as mature females and approximately 12% as immature animals. Male classified as immature animals were collected all months of the year except July, August, November and December. The average weight of correctly classified mature was 3556.1 ± 638.6g. Males classified as immature weighed significantly less, 2559.6 ± 258.1g (t-test: $p=3.53 \times 10^{-7}$). The average testis weight also differed significantly (t-test: $p=4.49 \times 10^{-9}$). The average testis weight for correctly classified mature males was 3.6 ± 1.4g, and for mature males classified as immature animals it was 1.0 ± 1.0g.

Mature males classified as mature females were collected at all times of the year. On average these males weighed 3116.7 ± 785.2g, which is significantly more than the mature males classified as immature (t-test: p=0.019) and less than correctly classified mature males (t-test: p=0.0097). The mean testes weight of mature males classified as mature females was 2.8 ± 1.6g, this is significantly more than the mature males classified as immature animals (t-test: p=0.0009) and significantly less than the correctly classified mature males (t-test: p= 0.025).

Principle factor analysis on the residuals showed that there is an underlying connection between all the histological parameters — the unrotated factor pattern (see Table 8) shows high correlations (≥0.4) for all histological parameters for the first common factor. When rotated the factor analysis identifies four factors (see Table 9). Factor 1 contains the three "depth" parameters (total tissue depth, holocrine sebaceous tissue depth and sudoriferous apocrine tissue depth). The second factor contains the tissue component parameters (ie. nuclear diameter, cell height and lumen diameter). The third and fourth factors contain one histological parameter each — percentage sudoriferous apocrine tissue and percentage holocrine sebaceous tissue respectively.

Table 8. Unrotated factor pattern of factor analysis on residuals.

Tissue Parameter (Studentised Residuals)	Factor 1	Factor 2	Factor 3	Factor 4
Total tissue depth	0.8	-0.5	0.1	0.3
HS tissue depth (square root transformed)	0.8	-0.4	0.3	0.0
Percent SA tissue	0.8	0.1	-0.5	-0.3
SA tissue depth	0.7	-0.4	-0.2	0.1
Percent HS tissue	0.7	0.2	0.4	-0.4
A lumen diameter	0.6	0.2	-0.1	0.0
SA nuclear diameter (log transformed)	0.4	0.6	0.0	0.4
SA cell height (log transformed)	0.4	0.5	-0.1	0.0
HS nuclear diameter	0.4	0.5	0.2	0.2
Variance explained	3.74	1.56	0.61	0.55

Correlations >0.4 (±) indicated by shading

Table 9. Rotated factor pattern of factor analysis on residuals.

Tissue Parameter (Studentised Residuals)	Factor 1	Factor 2	Factor 3	Factor 4
Total tissue depth	1.0	0.1	0.1	0.1
SA tissue depth	0.8	0.0	0.3	0.0
HS tissue depth (square root transformed)	0.8	0.1	0.1	0.5
SA nuclear diameter (log transformed)	0.1	0.8	0.1	0.0
HS nuclear diameter	0.1	0.7	0.0	0.2
SA cell height (log transformed)	0.0	0.6	0.4	0.2
SA lumen diameter	0.3	0.4	0.3	0.2
Percent HS tissue	0.3	0.2	0.9	0.2
Percent SA tissue	0.2	0.3	0.2	0.8
Variance explained	2.57	1.69	1.15	1.05

Correlations >0.4 (±) indicated by shading

Stepwise discriminant analysis (using the three gender-maturity groups) identified Factor 1 as the only important variable in group discrimination — Factor 1 ($F_{(2, 155)} = 36.446$, $P<0.001$). This factor incorporates the three tissue depth parameters. Pair-wise comparisons of the gender-maturity groups using Factor 1 from the factor analysis are shown below in Table 10. Factor 1 is able to separate all three gender-maturity groups. (NB: Immature males and females have been grouped together. Analysis with the immature animals separated by sex showed no significant difference between the sexes $p=0.63$).

Table 10. Discriminant (canonical variate) analysis: Pair-wise probabilities (based on squared differences / Mahalanobis distances) for gender-maturity group separation using Factor 1 identified from the factor analysis.

	Immature ♀ and ♂: Mature♀	Immature ♀ and ♂: Mature ♂	Mature ♀: Mature ♂
Factor 1	P<0.0001	P<0.0001	P<0.0001

The results of the cross-validation tests are shown in Table 11.

Table 11. Cross-validation results using a linear discriminant function. Percent classified into each group, based on Factor 1.

Group	Immature ♀ and ♂	Mature♀	Mature ♂	Total n
from Group				
Immature ♀ and ♂	73.91 (n=17)	26.09 (n=6)	0.0 (n=0)	23
Mature♀	18.75 (n=6)	53.13 (n=17)	28.13 (n=9)	32
Mature ♂	8.00 (n=8)	28.00 (n=28)	64.00 (n=64)	100
	31	51	73	155

Using the combined depth parameters of Factor 1 to separate the gender-maturity groups improved the percentage of animals correctly classified.

2.3.1.2. Males by maturity

Table 12 shows the results of the univariate analysis of the individual histological parameters. The following tissue parameters (shaded on table) showed significant differences between the gender-maturity groups: total tissue depth, holocrine sebaceous depth, sudoriferous apocrine depth, sudoriferous apocrine lumen diameter, percentage holocrine sebaceous tissue and percentage sudoriferous apocrine tissue. The male-maturity groups could not be significantly separated by the size of the cell nuclei for either tissue type, or by the height of the sudoriferous apocrine cells.

Table 12. Univariate test for male-maturity group separation.

Tissue Parameter	Transformation	F ratio	P
Total tissue depth	none	F(1, 116)=35.4487	P=0.0001
HS tissue depth	square root	F(1, 116)=29.6189	P=0.0001
SA tissue depth	none	F(1, 114)=15.2709	P=0.0001
HS nuclear diameter	none	F(1, 116)=0.3699	P=0.5443
SA nuclear diameter	natural logarithm	F(1, 106)<0.000	P=0.9992
SA cell height	natural logarithm	F(1, 106)=1.1546	P=0.2852
SA lumen diameter	none	F(1, 106)=8.5979	P=0.0041
Percent HS tissue	none	F(1, 11)=4.0035	P=0.0477
Percent SA tissue	none	F(1, 115)=4.7350	P=0.0316

Key: HS holocrine sebaceous
SA sudoriferous apocrine

As the comparison is between two groups, the p-values for the pair-wise comparisons of the tissue parameters are the same as the p-values given in the univariate analysis shown below in Table 10 above.

The mean values for each of the tissue parameters for the two groups is shown in Table 6 above (in *§ 2.3.1.1. Males and females by maturity*).

Stepwise canonical variate analysis showed that only one histological parameter is required to maximally separate the male-maturity groups, ie total tissue depth ($F_{(1, 116)}$=35.4487, P=0.0001).

2.3.1.3. Females by maturity

Table 13 shows the results of the univariate analysis of the individual histological parameters. The following tissue parameters (shaded on table) showed significant differences between the female-maturity groups: total tissue depth, holocrine sebaceous depth, sudoriferous apocrine depth, and sudoriferous apocrine lumen diameter. The female-maturity groups could not be significantly separated by the size of the cell nuclei for either tissue type, by the height of the sudoriferous apocrine cells, or by the percentage of holocrine sebaceous tissue and percentage sudoriferous apocrine tissue.

Table 13. Univariate test for female-maturity group separation.

Tissue Parameter	Transformation	F ratio	P
Total tissue depth	none	F(1, 50)=13.7248	P=0.0005
HS tissue depth	square root	F(1, 50)=16.6466	P=0.0002
SA tissue depth	none	F(1, 50)=12.0876	P=0.0011
HS nuclear diameter	none	F(1, 50)=0.0081	P=0.9288
SA nuclear diameter	natural logarithm	F(1, 45)=0.4539	P=0.5039
SA cell height	natural logarithm	F(1, 45)=0.1643	P=0.6871
SA lumen diameter	none	F(1, 45)=5.3101	P=0.0259
Percent HS tissue	none	F(1, 50)=1.2718	P=0.2648
Percent SA tissue	none	F(1, 50)=2.1571	P=0.1482

Key: HS holocrine sebaceous
SA sudoriferous apocrine

As the comparison is between two groups, the p-values for the pair-wise comparisons of the tissue parameters are the same as the p-values given in the univariate analysis shown below in Table 11 above.

The mean values for each of the tissue parameters for the two groups is shown in Table 6 above (in *§2.3.1.1. Males and females by maturity*).

Stepwise canonical variate analysis showed that only one histological parameter is required to maximally separate the female-maturity groups, ie total tissue depth ($F_{(1, 50)}$=13.7248, P=0.0005).

2.3.2. Gender by season

2.3.2.1. Mature males and females by season

Table 14 shows the results of the univariate analysis of the individual histological parameters. The following tissue parameters (shaded on table) showed significant differences between the gender-season groups: total tissue depth, holocrine sebaceous depth, and holocrine sebaceous nuclei diameter, and the percentage holocrine sebaceous tissue. (The gender-season groups could not be significantly separated by any of the sudoriferous apocrine tissue parameters, ie. tissue depth, lumen diameter, percentage of tissue, the size of the cell nuclei, or the height of the cells.)

Table 14. Univariate test for gender-season group separation.

Tissue Parameter	Transformation	F ratio	P
Total tissue depth	none	F(7, 135)=2.1714	P=0.0405
HS tissue depth	square root	F(7, 135)=2.3519	P=0.0268
SA tissue depth	none	F(7, 134)=1.2213	P=0.2953
HS nuclear diameter	none	F(7, 135)=2.7812	P=0.0098
SA nuclear diameter	natural logarithm	F(7, 124)=0.5189	P=0.8189
SA cell height	natural logarithm	F(7, 124)=1.4010	P=0.2107
SA lumen diameter	none	F(7, 124)=08213	P=0.5713
Percent HS tissue	none	F(7, 135)=2.9264	P=0.0070
Percent SA tissue	none	F(7, 134)=1.3898	P=0.2146

Key: HS holocrine sebaceous
SA sudoriferous apocrine

Pair-wise comparisons of the tissue parameters shown to be significant in the univariate analysis are shown below in Tables 15.

(NOTE: Only the results for the comparing males and females are shown here. The results of this analysis for males by season and females by season are shown below with the results of the individual sex by season analyses.

The mean values for each of the tissue parameters for each of the groups is shown in Table 16.

Table 15. Pair-wise squared differences between gender-season groups from single variable stepwise discriminant (canonical variate) analysis.

Tissue Parameter	♂Pre-breeding: ♀Pre-breeding	♂Breeding: ♀Breeding	♂Post-breeding: ♀Post-breeding	♂Dispersion: ♀Dispersion
Total tissue depth	P=0.4041	P=0.0171	P=0.0692	P=0.0661
HS tissue depth (square root transformed)	P=0.4665	P=0.0105	P=0.0694	P=0.0273
HS nuclear diameter	P=0.4658	P=0.1756	P=0.3647	P=0.8277
Percent HS tissue	P=0.4792	P=0.0746	P=0.0323	P=0.2316

Tissue Parameter	♂Pre-Breeding: ♀Breeding	♂Pre-Breeding: ♀Post-Breeding	♂Pre-Breeding: ♀Dispersion	♂Breeding: ♀Post-Breeding	♂Breeding: ♀Dispersion	♂Post-Breeding: ♀Dispersion
Total tissue depth	P=0.1094	P=0.0387	P=0.1077	P=0.0052	P=0.0314	P=0.1485
HS tissue depth (square root transformed)	P=0.0230	P=0.0501	P=0.0178	P=0.0256	P=0.0102	P=0.0232
HS nuclear diameter	P=0.9116	P=0.1813	P=0.0514	P=0.1538	P=0.0443	P=0.6677
Percent HS tissue	P=0.0068	P=0.0205	P=0.0066	P=0.1488	P=0.0367	P=0.0094

Tissue Parameter	♀Pre-Breeding: ♂Breeding	♀Pre-Breeding: ♂Post-Breeding	♀Pre-Breeding: ♂Dispersion	♀Breeding: ♂Post-Breeding	♀Breeding: ♂Dispersion	♀Post-Breeding: ♂Dispersion
Total tissue depth	P=0.1520	P=P=0.5028	P=0.2674	P=0.1779	P=0.0586	P=0.0205
HS tissue depth (square root transformed)	P=0.3383	P=0.5157	P=0.5442	P=0.0351	P=0.0453	P=0.0842
HS nuclear diameter	P=0.2047	P=0.0038	P=0.0191	P=0.0433	P=0.1986	P=0.9512
Percent HS tissue	P=0.9858	P=0.5406	P=0.3736	P=0.0123	P=0.6199	P=0.8081

Significant differences (P≤0.05) are shaded

Table 16. Tissue parameter means (μm) and standard errors.

Tissue Parameter	♂ Pre-Breeding	Breeding	Post-Breeding	Dispersion	♀ Pre-breeding	Breeding	Post-breeding	Dispersion
Total tissue depth	1700 (± 69.6)	1810 (±91.7)	1673 (± 75.7)	1758 (± 89.4)	1550 (± 119.0)	1486 (± 61.5)	1406 (± 106.5)	1386 (± 193.0)
HS tissue depth (square root transformed)	24.9 (± 0.9)	25.4 (±1.1)	24.8 (± 0.8)	24.7 (± 0.9)	23.4 (± 1.1)	21.4 (± 1.0)	21.7 (±1.2)	19.6 (±2.4)
HS tissue depth (reverse transformed)	643.7	676.3	627.7	626.6	554.6	470.4	485.9	411.4
HS nuclear diameter	9.1 (±0.2)	9.1 (± 0.2)	8.4 (± 0.2)	8.6 (± 0.2)	9.7 (± 0.2)	9.1 (± 0.3)	8.6 (± 0.4)	8.1 (± 0.4)
Percent HS tissue	16.1 (± 1.2)	14.4 (±1.1)	15.9 (±0.9)	12.1 (±0.8)	14.4 (±1.3)	11.2 (± 1.3)	11.6 (±1.6)	8.9 (± 2.3)

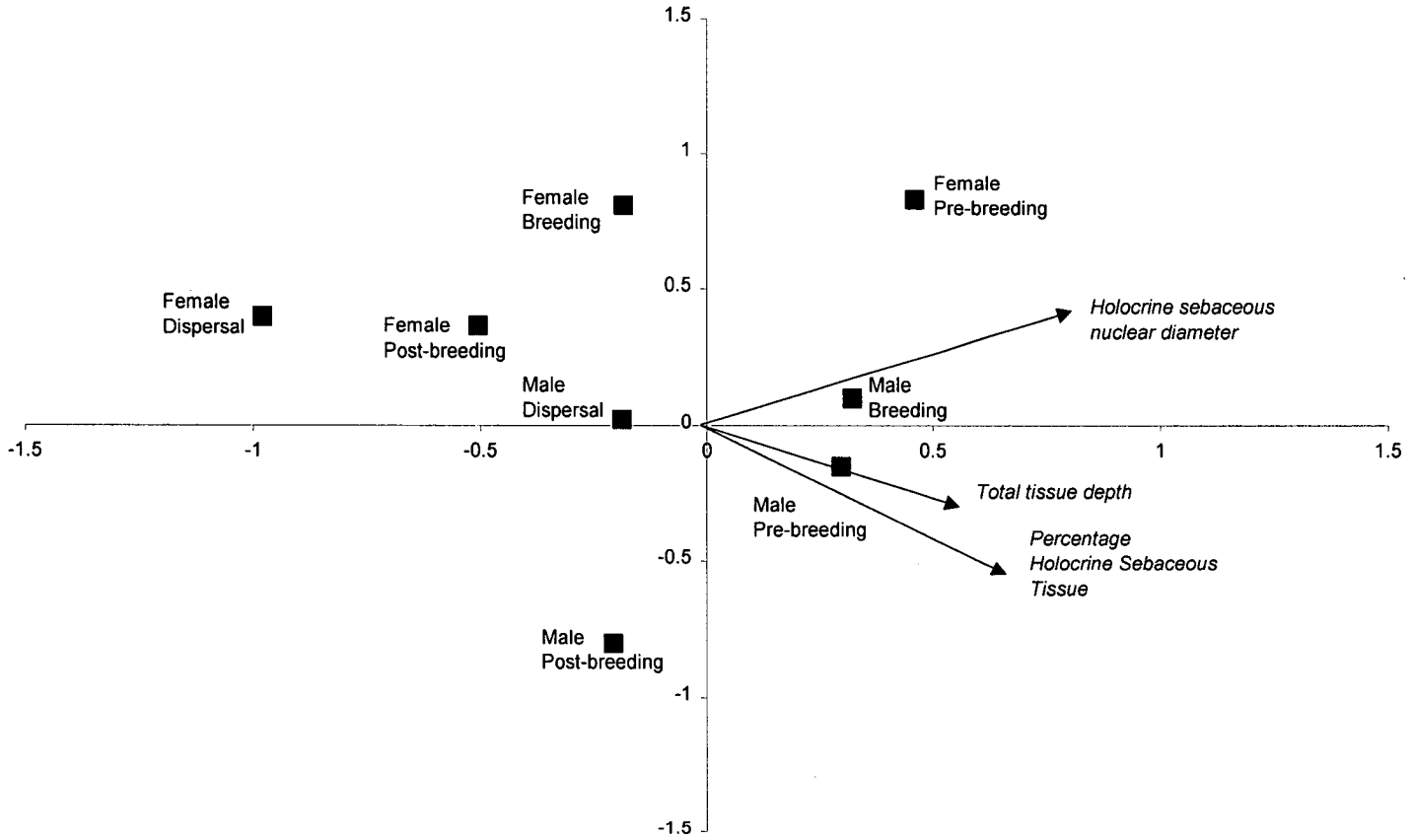

Figure 2. Canonical variate plot of gender-season groups.

Significant differences between mature males and females were not seen in every season or for all parameters during a particular season. There were no significant differences between males and females in the pre-breeding season. In the breeding season males had a significantly greater total tissue depth and holocrine sebaceous tissue depth than females. Holocrine sebaceous depth was also greater in males than females during the dispersion period. Total tissue depth was greater in males than females at this time, but was not significant at the 0.05 level. In the post breeding season males had a significantly greater percentage of holocrine sebaceous tissue than females.

Stepwise canonical variate analysis showed that three histological parameters are required to maximally separate the gender-season groups, see Table 17. The results of the "pooled within canonical structure" are also shown in this table

Table 17. Stepwise discriminant analysis.

Tissue Parameter	R^2	F ratio	P	Canonical variate 1	Canonical variate 2
HS nuclear diameter	0.1260	2.7812	0.0098	0.421921	0.88351
Percent HS tissue	0.1318	2.9264	0.0070	-0.546528	0.728322
Total tissue depth	0.1012	2.1714	0.0405	-0.300955	0.616507

Figure 2 shows the position of each gender-season group based on the canonical variate analysis.

2.3.2.2. Mature males by season

Table 18 shows the results of the univariate analysis of the individual histological parameters. Only holocrine sebaceous nuclear diameter (shaded on table) was significantly different between the mature male-season groups. The pair-wise comparisons are shown in Table 19.

The mean values for each of the tissue parameters for each of the male-season groups is shown in Table 16 in part 1 above. NOTE: in the univariate analysis of male by season only the holocrine sebaceous nuclear diameter was significant.

The nuclear diameters for holocrine sebaceous tissue were significantly greater in the pre-breeding and breeding seasons than in the post-breeding season. Although not significant the nuclear diameter in the dispersion period was greater than in the post-breeding period, but not as high as during the pre-breeding and breeding seasons.

Stepwise canonical variate analysis showed that two histological parameters are required to maximally separate the male-season groups, see Table 20. The results of the "pooled within canonical structure" are also shown in this table.

Table 18. Univariate test for mature male-season group separation.

Tissue Parameter	Transformation	F ratio	P
Total tissue depth	none	F(3, 104)=0.5735	P=0.6337
HS tissue depth	square root	F(3, 104)=0.1120	P=0.9529
SA tissue depth	none	F(3, 103)=0.5171	P=0.6714
HS nuclear diameter	none	F(3, 104)=3.7142	P=0.0139
SA nuclear diameter	natural logarithm	F(3, 96)=1.0849	P=0.3593
SA cell height	natural logarithm	F(3, 96)=0.5709	P=0.6355
SA lumen diameter	none	F(3, 96)=1.2603	P=0.2924
Percent HS tissue	none	F(3, 104)=2.5388	P=0.0606
Percent SA tissue	none	F(3, 103)=1.0244	P=0.3851

Key: HS holocrine sebaceous
SA sudoriferous apocrine

Table 19. Pair-wise squared differences between mature male-season groups from single variable stepwise discriminant (canonical variate) analysis.

A: results of the analysis comparing both sexes with season (see Part 1 above).
B: results of the analysis of males by season.

A.

Tissue Parameter	Pre-Breeding: Breeding	Pre-Breeding: Post-Breeding	Pre-Breeding: Dispersion	Breeding: Post-breeding	Breeding: Dispersion	Post-Breeding: Dispersion
Total tissue depth	P=0.2864	P=0.8025	P=0.6109	P=0.2130	P=0.6516	P=0.4791
HS tissue depth (square root transformed)	P=0.6804	P=0.9148	P=0.8774	P=0.6227	P=0.6030	P=0.9602
HS nuclear diameter	P=0.8881	P=0.0068	P=0.0818	P=0.0052	P=0.0655	P=0.4154
Percent HS tissue	P=0.2281	P=0.8906	P=0.0105	P=0.3190	P=0.1411	P=0.0208

B.

Tissue Parameter	Pre-Breeding: Breeding	Pre-Breeding: Post-Breeding	Pre-Breeding: Dispersion	Breeding: Post-breeding	Breeding: Dispersion	Post-Breeding: Dispersion
HS nuclear diameter	P=0.0710	P=0.0080	P=0.0879	P=0.0062	P=0.8901	P=0.4241
Percent HS tissue*	P=0.2411	P=0.8935	P=0.0130	P=0.3322	P=0.1523	P=0.0248

Significant differences (P≤0.05) are shaded
*NOTE: Although percent HS tissue is not significant (p=0.0606) in univariate test of males by season, it is shown here because as it was close to being significant and the significant pairwise comparisons match those found in the gender by sex analysis where the percent HS tissue was significant (P=0.0070)

Table 20. Stepwise discriminant analysis.

Tissue Parameter	R^2	F ratio	P	Canonical variate 1	Canonical variate 2
HS nuclear diameter	0.96771	3.7142	0.0139	-0.677422	0.735595
Percent HS tissue	0.068237	2.5388	0.0606	0.305795	0.952097

Figure 3 shows the position of each gender-season group based on the canonical variate analysis.

Trends in mature male by season data for all the holocrine sebaceous data are shown in Figure 4 and for the sudoriferous apocrine data in Figure 5.

The greatest depths of holocrine sebaceous tissue and the largest holocrine sebaceous nuclei diameters are seen during the breeding and dispersion periods in males. The greatest percentage of holocrine sebaceous tissue is seen in the periods proceeding the breeding and dispersion seasons.

Trends in the sudoriferous apocrine tissue appear more complicated. As with the holocrine sebaceous tissue, the greatest tissue depth and largest nuclear diameters are found in the breeding and dispersion periods. The cell height, lumen diameter and percentage of sudoriferous apocrine tissue are smallest during the breeding season. Cell height is fairly constant during the year, showing a decrease during the breeding season. Lumen diameters are greatest during the dispersion and pre-breeding periods. And the greatest percentage of apocrine tissue is seen in the post-breeding season.

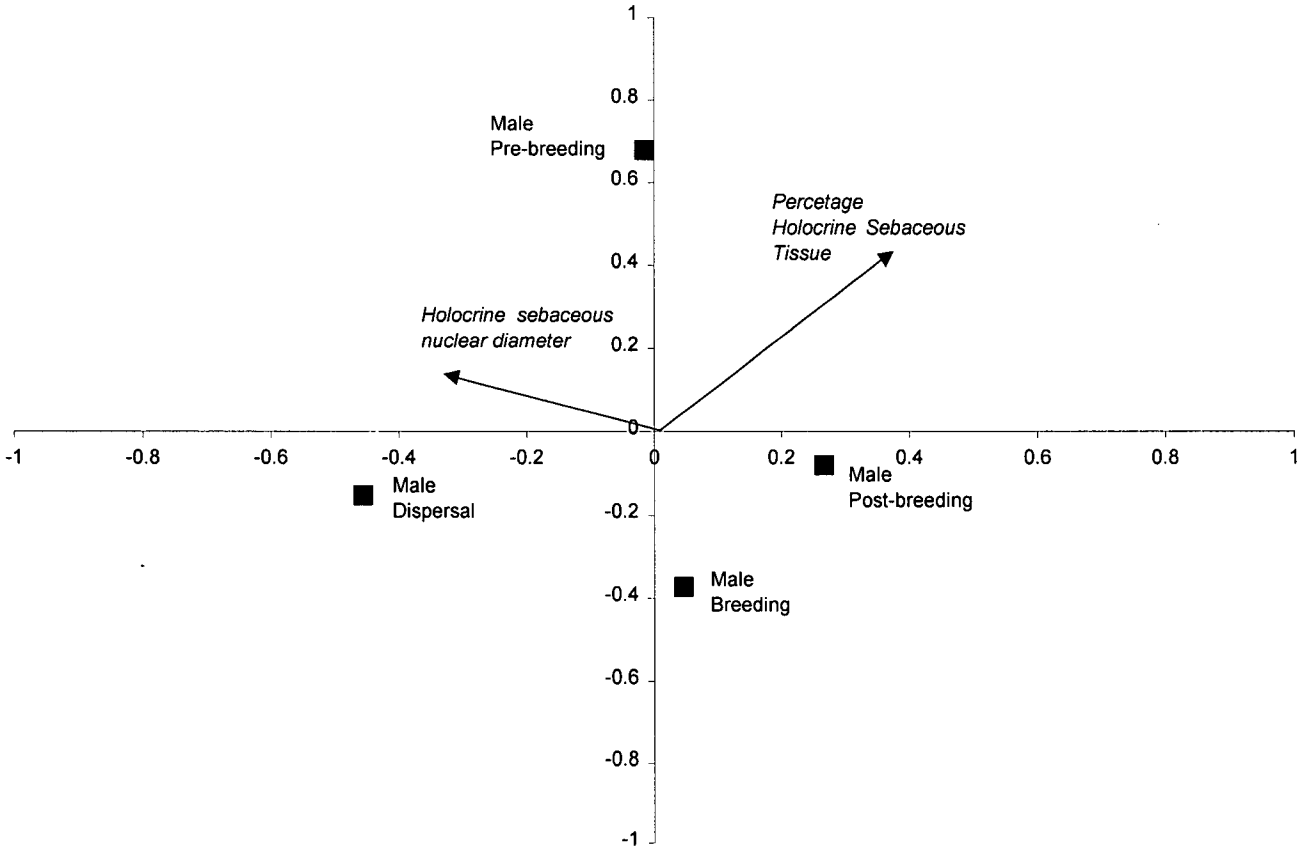

Figure 3. Canonical variate plot of mature males by season.

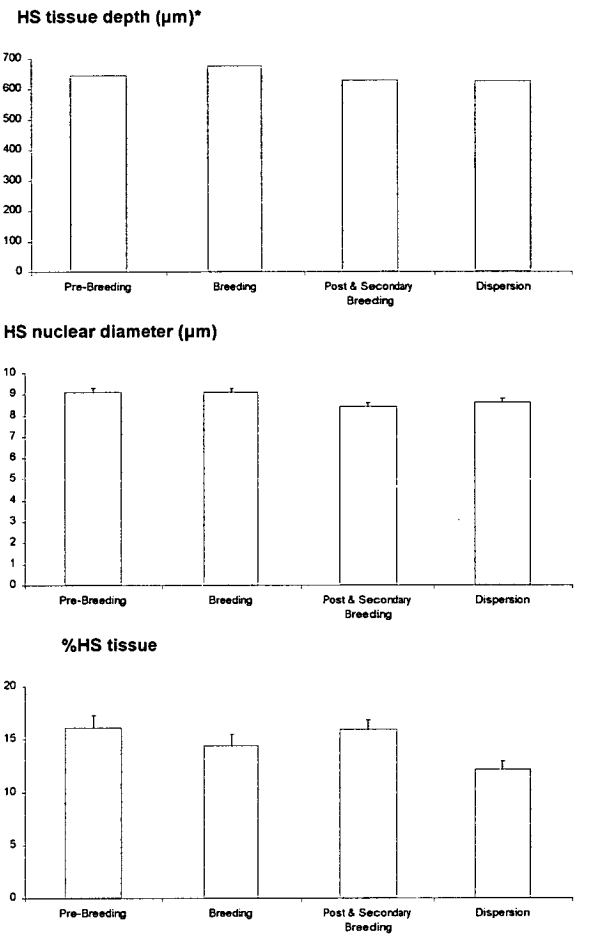

Figure 4. Trends in male holocrine sebaceous (HS) tissue parameters over four seasons.
* no error bars, data have been reverse transformed.

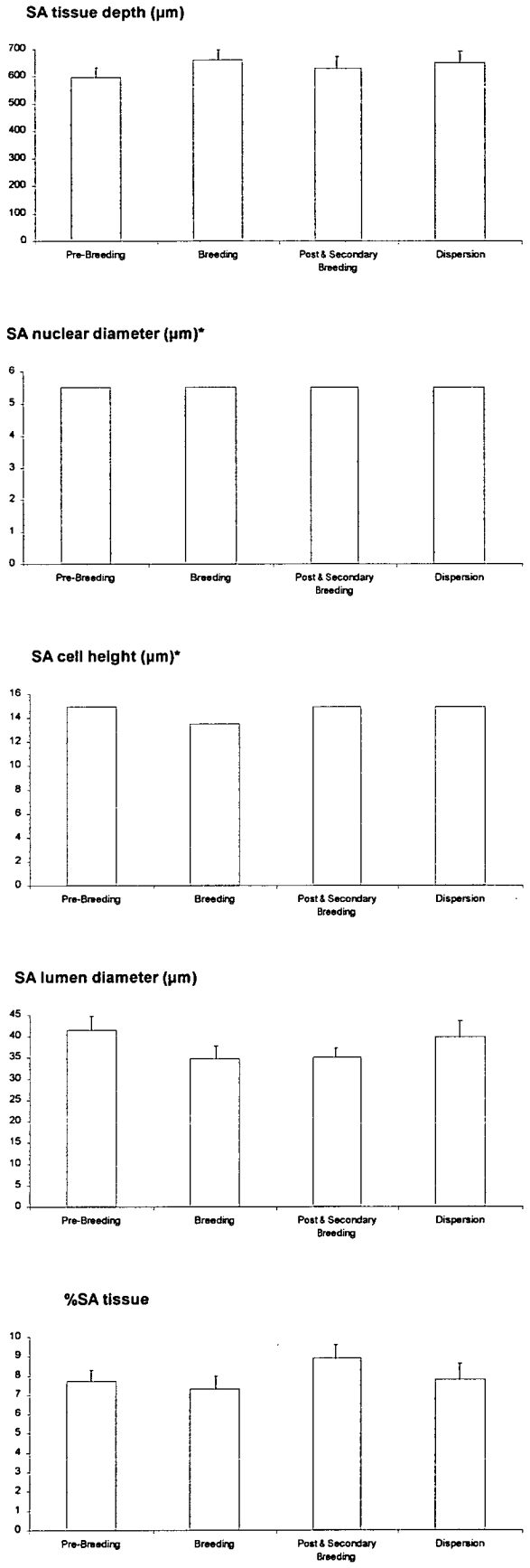

Figure 5. Trends in male sudoriferous apocrine (SA) tissue parameters over four seasons.
* no error bars, data have been reverse transformed.

2.3.2.3. Mature females by season

Table 21 shows the results of the univariate analysis of the individual histological parameters. Only holocrine sebaceous nuclear diameter (shaded on table) was significantly different between the mature female-season groups. The pair-wise comparisons are shown in Table 22.

Table 21. Univariate test for mature female-season group separation.

Tissue Parameter	Transformation	F ratio	P
Total tissue depth	none	F(3, 31)=0.4013	P=0.7530
HS tissue depth	square root	F(3, 31)=0.9433	P=0.4316
SA tissue depth	none	F(3, 31)=0.0208	P=0.9935
HS nuclear diameter	none	F(3, 31)=2.9653	P=0.0472
SA nuclear diameter	natural logarithm	F(3, 28)=0.1475	P=0.9304
SA cell height	natural logarithm	F(3, 28)=2.1339	P=0.1184
SA lumen diameter	none	F(3, 28)=0.4668	P=0.7078
Percent HS tissue	none	F(3, 31)=1.1798	P=0.3334
Percent SA tissue	none	F(3, 31)=0.0832	P=0.9687

Key: HS holocrine sebaceous
SA sudoriferous apocrine

Table 22. Pair-wise squared differences between mature female-season groups from single variable stepwise discriminant (canonical variate) analysis.

A: results of the analysis comparing both sexes with season (see Part 1 above).
B: results of the analysis of females by season.

A.

Tissue Parameter	Pre-Breeding: Breeding	Pre-Breeding: Post-Breeding	Pre-Breeding: Dispersion	Breeding: Post-Breeding	Breeding: Dispersion	Post-Breeding: Dispersion
Total tissue depth	P=0.7499	P=0.4827	P=0.5032	P=0.6270	P=0.6372	P=0.9274
HS tissue depth (square root transformed)	P=0.3835	P=0.4721	P=0.1732	P=0.8731	P=0.4512	P=0.3920
HS nuclear diameter	P=0.1953	P=0.0359	P=0.0115	P=0.2923	P=0.0866	P=0.3797
Percent HS tissue	P=0.2350	P=0.3226	P=0.0972	P=0.8362	P=0.4235	P=0.3486

B.

Tissue Parameter	Pre-Breeding: Breeding	Pre-Breeding: Post-Breeding	Pre-Breeding: Dispersion	Breeding: Post-Breeding	Breeding: Dispersion	Post-Breeding: Dispersion
HS nuclear diameter	P=0.1749	P=0.0309	P=0.0103	P=0.2680	P=0.0751	P=0.3544

Significant differences (P≤0.05) are shaded

The mean values for each of the tissue parameters for each of the female-season groups is shown in Table 14 in part 1 above. NOTE: in the univariate analysis of female by season only the holocrine sebaceous nuclear diameter was significant.

The nuclear diameters for holocrine sebaceous tissue were significantly greater in the pre-breeding than either the post-breeding or the dispersion season. Although not significant the nuclear diameter in the breeding period was also greater than either the post-breeding or the dispersion season, but not as high as during the pre-breeding period.

Stepwise canonical variate analysis showed that one histological parameter is required to maximally separate the female-season groups, ie holocrine sebaceous nuclear diameter ($F_{(3, 31)}$=2.9653, P=0.0472).

Trends in mature female by season data for all the holocrine sebaceous data are shown in Figure 6 and for the sudoriferous apocrine data in Figure 7.

The greatest depths of holocrine sebaceous tissue, the largest holocrine sebaceous nulcei diameters and the greatest percentage of holocrine sebaceous tissue are seen during the pre-breeding period in females. At all other times of the year these three parameters are not as great.

The sudoriferous apocrine tissue parameters do not show any clear trends. Sudoriferous apocrine tissue depth, nuclear diameters and the percentage of sudoriferous apocrine tissue vary only slightly between the four seasons. Cell height and lumen diameters are greatest in the pre-breeding and dispersion periods.

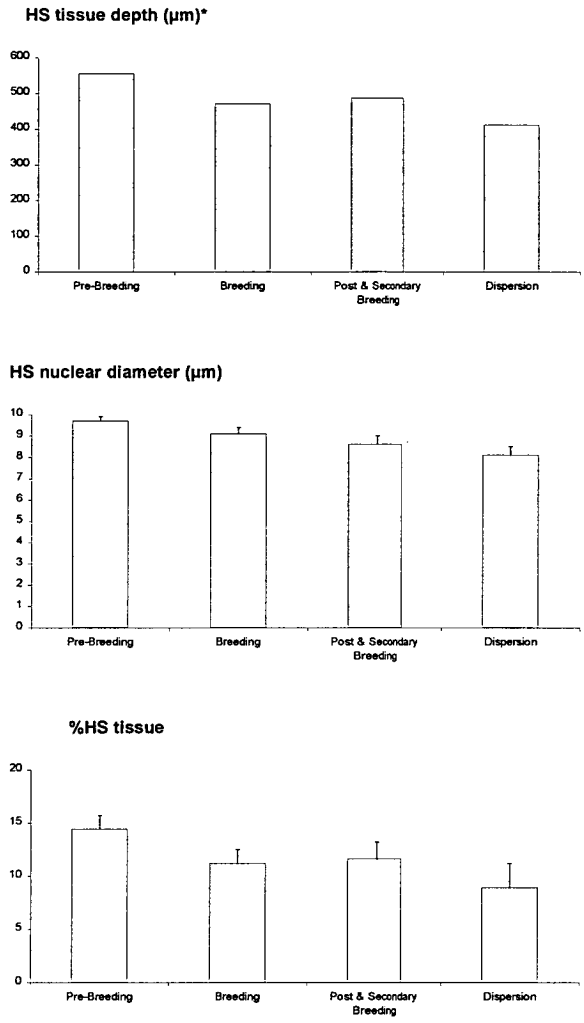

Figure 6. Trends in female holocrine sebaceous (HS) tissue parameters over four seasons.
* no error bars, data have been reverse transformed.

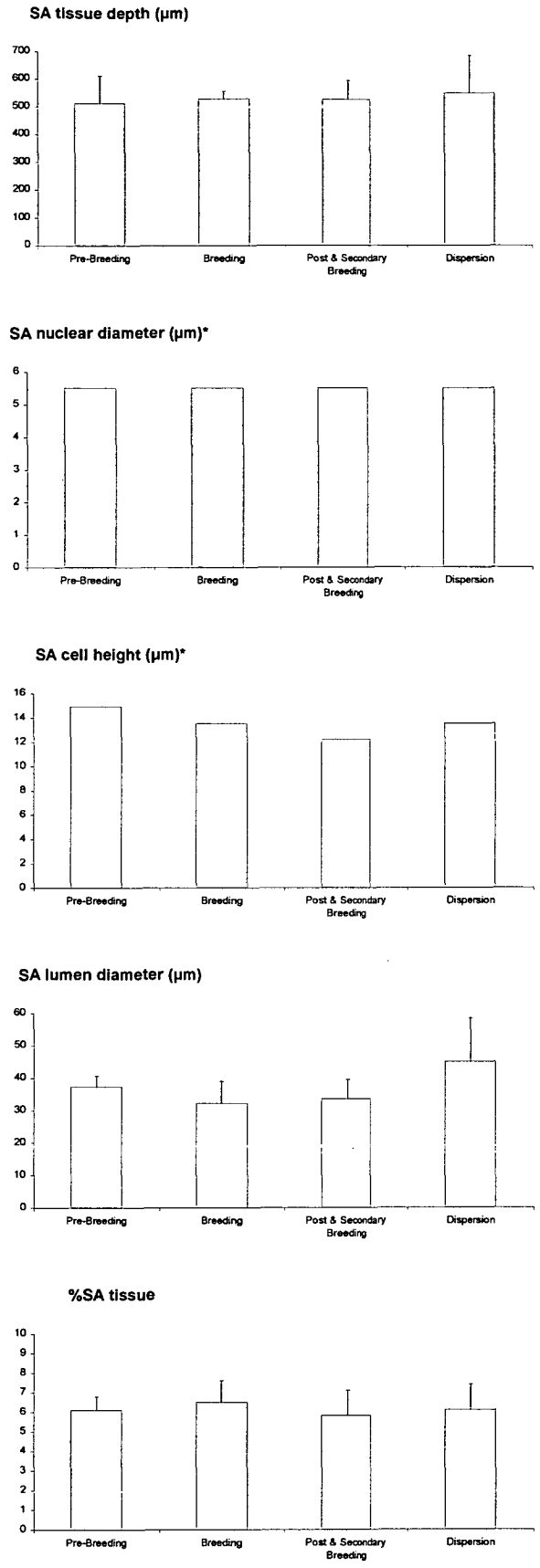

Figure 7. Trends in female sudoriferous apocrine (SA) tissue parameters over four seasons.

* no error bars, data have been reverse transformed.

2.3.3. Females by reproductive status

2.3.3.1. Immature and mature females by reproductive status

Table 23 shows the results of the univariate analysis of the individual histological parameters. The following tissue parameters showed significant differences between the female-maturity groups: total tissue depth, holocrine sebaceous depth, and sudoriferous apocrine tissue depth. (The female-maturity groups could not be significantly separated by the diameter of the nuclei of either tissue type, the percentage of either tissue type, or by the height or lumen diameter of the sudoriferous apocrine cells.)

Table 23. Univariate test for female-maturity group separation.

Tissue Parameter	Transformation	F ratio	P
Total tissue depth	none	F(3, 48)=4.4232	P=0.0080
HS tissue depth	square root	F(3, 48)=5.8466	P=0.0017
SA tissue depth	none	F(3, 48)=4.8133	P=0.0052
HS nuclear diameter	none	F(3, 48)=1.0659	P=0.3724
SA nuclear diameter	natural logarithm	F(3, 43)=0.5167	P=0.6730
SA cell height	natural logarithm	F(3, 43)=1.4743	P=0.2349
SA lumen diameter	none	F(3, 43)=1.7402	P=0.1730
Percent HS tissue	none	F(3, 48)=1.1238	P=0.3488
Percent SA tissue	none	F(3, 48)=1.3898	P=0.4791

Key: HS holocrine sebaceous
SA sudoriferous apocrine

Pair-wise comparisons of the tissue parameters shown to be significant in the univariate analysis are shown below in Tables 24.

There were no significant differences between the three groups of mature females for any of the tissue parameters. The mean values for each of the tissue parameters for each of the groups is shown in Table 25. The only significant differences among females of different reproductive status were between immature females and each of the three mature groups. Immature females had significantly less tissue depth (ie total, holocrine sebaceous and sudoriferous apocrine) then all three of the mature female groups. The only exception was the sudoriferous apocrine tissue depth of anoestrus females, which was not significantly different to immature females.

Table 24. Pair-wise squared differences between female-maturity groups from single variable stepwise discriminant (canonical variate) analysis.

Tissue Parameter	Immature: Anoestrus	Immature: Oestrus	Immature: Pouch Young	Anoestrus: Oestrus	Anoestrus: Pouch Young	Oestrus: Pouch Young
Total tissue depth	P=0.0209	P=0.0331	P=0.0014	P=0.8716	P=0.9429	P=0.7876
HS tissue depth (square root transformed)	P=0.0019	P=0.0504	P=0.0006	P=0.2850	P=0.5250	P=0.4973
SA tissue depth	P=0.1750	P=0.0026	P=0.0026	P=0.1375	P=0.3425	P=0.3772

Significant differences (P≤0.05) are shaded

Table 25. Tissue parameter means (μm) and standard errors.

Tissue Parameter	Immature	Anoestrus	Oestrus	Pouch Young
Total tissue depth	1127 (± 73)	1457 (±114)	1430 (± 98)	1467 (± 72)
HS tissue depth (square root transformed)	17.3 (± 0.7)	22.6 (±1.6)	20.5 (± 1.6)	21.6 (± 0.8)
HS tissue depth (reverse transformed)	308.9	528.9	438.7	478.9
SA tissue depth	341.3 (±34.3)	452.6 (± 75.2)	597.9 (± 80.3)	527.9 (± 41.6)

Stepwise canonical variate analysis showed that two histological parameters are required to maximally separate the female-maturity groups, see Table 26. The results of the "pooled within canonical structure" are also shown in this table.

Differences between immature and mature females are related to both holocrine sebaceous and sudoriferous apocrine tissue depth. This differs slightly from the earlier analysis of females maturity (§ 2.3.1.3) were total tissue depth alone separated the mature and immature females.

Table 26. Stepwise discriminant analysis.

Tissue Parameter	R^2	F ratio	P	Canonical variate 1	Canonical variate 2
HS tissue depth (square root transformed)	0.267621	F=5.8466	P=0.0017	0.972795	-0.231668
SA tissue depth	0.231259	F=4.8133	P=0.0052	0.782755	0.622329

Figure 8 shows the position of each female-reproductive status group based on the canonical variate analysis.

It is important to note that the separation visible between the mature females is being greatly influenced by the immature animals. To see if there are any significant differences between the mature reproductive groups the analysis was repeated without the immature individuals in the following section.

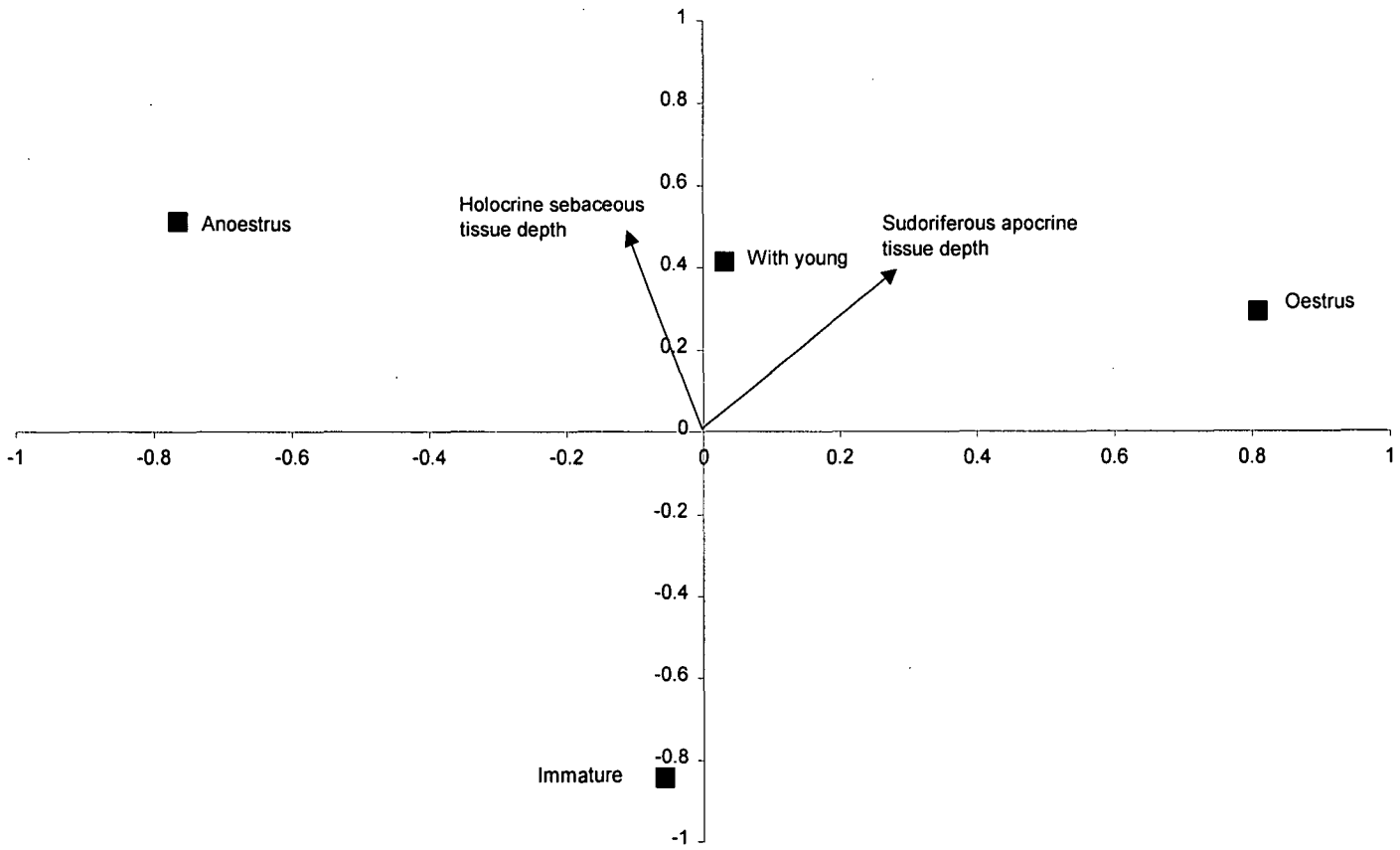

Figure 8. Canonical variate plot of females by reproductive state.

2.3.3.2. Mature females by reproductive status

Table 27 shows the results of the univariate analysis of the individual histological parameters. There were no significant differences between the tissue parameters of the three mature female groups.

Stepwise canonical variate analysis showed that there was no histological parameter/s that could be used to significantly separate the groups of mature females.

Table 27. Univariate test for mature female-reproductive state group separation.

Tissue Parameter	Transformation	F ratio	P
Total tissue depth	none	F(2, 32)=0.0358	P=0.9649
HS tissue depth	square root	F(2, 32)=0.5084	P=0.6062
SA tissue depth	none	F(2, 32)=0.9581	P=0.3944
HS nuclear diameter	none	F(2, 32)=1.5101	P=0.2298
SA nuclear diameter	natural logarithm	F(2, 29)=0.5257	P=0.5966
SA cell height	natural logarithm	F(2, 29)=2.1927	P=0.1298
SA lumen diameter	none	F(2, 32)=1.0590	P=0.3586
Percent HS tissue	none	F(2, 32)=0.2198	P=0.8039
Percent SA tissue	none	F(2, 29)=0.0503	P=0.9510

Key: HS holocrine sebaceous
SA sudoriferous apocrine

Despite the lack of significant results it is useful to look at the descriptive statistics for these three groups of females. Table 28 gives the mean values for each of the tissue parameters for each reproductive state.

Table 28. Tissue parameter means (μm) and standard errors.

Tissue Parameter	Anoestrus	Oestrus	Pouch Young
Total tissue depth	1457 (±114)	1430 (± 98)	1467 (± 72)
HS tissue depth (square root transformed)	22.6 (±1.6)	20.5 (± 1.6)	21.6 (± 0.8)
(reverse transformed)	528.9	438.7	478.9
SA tissue depth	452.6 (± 75.2)	597.9 (± 80.3)	527.9 (± 41.6)
HS nuclear diameter	9.4 (±0.3)	8.4 (±0.4)	8.9 (±0.2)
SA nuclear diameter (natural logarithm transformed)	1.7 (±0.06)	1.6 (±0.05)	1.7 (±0.04)
(reverse transformed)	5.5	5.0	5.5
SA cell height (natural logarithm transformed)	2.7 (± 0.04)	2.5 (± 0.06)	2.6 (± 0.05)
(reverse transformed)	14.9	12.2	13.5
SA lumen diameter	33.4 (± 8.6)	35.2 (± 5.4)	36.4 (± 5.4)
Percent HS tissue	13.8 (±2.0)	11.8 (± 2.7)	10.7 (± 0.9)
Percent SA tissue	6.5(± 1.5)	6.8 (± 0.5)	5.8 (± 1.3)

The greatest depths of holocrine sebaceous tissue and the largest holocrine sebaceous nuclei diameters and the greatest percentage of holocrine sebaceous tissue are seen in females in anoestrus condition. The sudoriferous apocrine tissue parameters as a group do not show any clear trend. Sudoriferous apocrine tissue depth is greatest in females in oestrus. The cell height and nuclear diameters, however, are lowest in females in oestrus. Sudoriferous apocrine tissue percentage and lumen diameters do not vary greatly between the three reproductive states.

2.4. Discussion

A range of differences in the histology of the sternal gland between and within the sexes, between mature and immature animals, and between groups of possums over different seasons was found. Many of these differences correlate well with what is already known about the biology and olfactory communication of the brushtail possum. The findings are also supported by work on other mammals.

2.4.1. Gender and maturity

Comparison of sexually mature and immature possums reveals that the development of the sternal gland is related to the onset of sexual maturity. Tissue parameters in immature males and females were not significantly different. Mature animals, however, had significantly greater tissue development than immature animals. The total depth of glandular tissue and the depth of each glandular type were greater in mature animals; the lumen diameters were also greater in mature animals; and mature males had a greater percentage of both tissue types compared to immature animals. Differences in the depth of the holocrine sebaceous tissue alone, followed closely by total tissue depth were great enough to separate immature and mature animals when the sexes were examined together. When the sexes were considered separately, differences in total tissue depth alone were able to separate immature males from mature males and immature females from mature females. Principle factor analysis revealed that one factor containing the three measurements of tissue depth was effective in separating the possums on the basis of gender and maturity.

Development of odour producing glands as an animal reaches sexual maturity is seen in a number of mammals (Johnson 1973; Brown 1979; Stoddart 1980b), including the rabbit (Mykytowycz 1965, 1966a & b). In the brushtail possum, Bolliger and Hardy (1944) observed that the area of brown fur on the sternum becomes increasingly obvious as possums become sexually mature. Winter (1977) observed that scent marking was not usually performed by juveniles, although a female as young as 8 ½ months was seen "chesting". (It should be noted that the first oestrus cycling has been recorded in females as young as 9 months (Pilton & Sharman 1962), although this is not usual). Juvenile males were not observed to mark until 21 months of age, although the difference in the age of the sexes may be due to lack of observations of juvenile males. Winter did note, however, that no juvenile males were seen marking until after they had left the maternal home range. In a series of experiments Bolliger (1944a & b) demonstrated that development of the brown fur on the sternum in juvenile male possums (6 months of age) could be prevented by castration. Subsequent administration of testosterone propionate resulted in sternal hairs developing the typical brown colour over an area equivalent to those seen in intact males of the same age. Removal of the ovaries in immature females (8-11 months) also prevented the development of the "sternal streak". Administration of oestradiol propionate did not change the appearance of the sternal region, although it did result in secretion and staining around the pouch. Development of the sternal hairs and secretion could be induced in overectomised females by administering testosterone propionate. The sternal region of female treated in this way showed the same level of development as mature males.

Cross validation analysis revealed that not all animals were classified in the group to which they were assigned. The four immature females that were classified as mature females were probably young females approaching sexual maturity and their first period of oestrus cycling. All were collected in the pre-breeding period between January and March. As in the mountain possum, *Trichosurus caninus,* (Smith & How 1973; How 1976), females without pouches and inverted nipples are not considered to be sexually mature (Hocking

1981). Around the time when a female first comes into oestrus during her first breeding season, the pouch forms and contains moist secretion, the nipples increase in size and the mammary glands become "palpable" (Bolliger & Carrodus 1938b, 1940). Further evidence that these females may have been approaching maturity is provided by their body weight. Although not significantly different, the weight of the four females classified as mature was higher than the correctly classified immature females.

The immature male classified as a mature female is an interesting case. This individual had well-developed sternal tissue compared to other juvenile males of the same weight and level of testicular development. The area of staining on the sternum was no greater than in other immature males, however. Given that this animal was collected in October it is possible that he had dispersed from the maternal home range and was independent. This male may have found habitat that was not already occupied by another male. The subsequent lack of competition may have precipitated the development of sternal tissue, enabling the young male to mark and "claim" the area as his own. Young males "in the process of establishing themselves" have been observed to mark their habitat (Winter 1977).

Examination of the histology of the sternal gland in immature and mature brushtail possums supports previous findings that the sternal gland shows its greatest development in sexually mature animals.

Comparison of the histology of mature possums reveals differences between the sexes. The differences are in the scale of the glandular tissue. Males had greater glandular tissue depths and a higher percentage of each tissue type. There were no significant differences in the size of the cell nuclei, cell height or lumen diameters. The greater amount of glandular tissue in males is supported by a range of observations of mature possums. Externally the development of the sternal gland is greater in males than females (Bolliger & Hardy 1944) and males are known to mark with the sternal gland more often than females (Winter 1977). The range of chemical compounds found in secretions from the sternal gland is also greater in males (Biggins 1979; Salamon 1994, 1998).

Differences in scent glands between the sexes have been found in other mammals. The sternal gland of male North American opossums *(Didelphis virginiana)* shows greater morphological and histological development and secretory activity than in females (Meisner 1986).

As in the immature animals, misclassification occurred among the mature males and females. The 30% misclassification of mature males appears to be related to size. There are significant differences in body weight and testis size between correctly classified mature males and mature males classified as mature females and immature animals. Although the body weights of the mature males classified as immature were greater than the 2000g body weight used as the cut off point for maturity in this study, their testis weight was low. These males had very small testes and using Gilmore's observation, that spermatozoa were rarely found in males with a testis weight <2g, these males would have been classified as immature. The males classified as mature females had body weights and testis weights in between those of the mature males classified as immature and mature.

Misclassification may be related to the season in which the male was collected. Seasonal variation in the amount of sternal gland marking performed by mature males has been observed, with peaks during times of sexual activity (Winter 1977). There may be some regression of the sternal gland tissue in mature males during periods of low marking frequency which results in it appearing more like that of a mature females or an immature individual. This explanation seems unlikely, however. No seasonal variation in testes size or spermatogenesis in mature males occurs (Bolliger & Carrodus 1938a; Bolliger 1942, 1946; Tyndale-Biscoe 1955; Dunnet 1956; Gilmore 1969). And although seasonal variations in prostate size (Gilmore 1969), plasma testosterone and body weight have been recorded in male possums, with a peak in March and a nadir in September (Gemmell *et al*

1986) and testosterone has been shown to influence the development and maintenance of the sternal gland (Bolliger 1944a & b; Biggins 1979), there is no relationship between the group the mature males were assigned to and season. Mature males classified as females were collected at all times of the year and those classified as immature during most months.

Misclassification of the mature males may be related to the sample of roadkills collected. Animals in this study were collected over a wide geographical range and it is possible that there are some differences in the relative size at which males reach maturity. Hocking (1981) found that the age at which sexual maturity was reached was associated with the condition of the habitat occupied by a population. The condition of the habitat of possums collected in this study was impossible to ascertain given that the animals were collected from roadsides. It is known that a latitudinal gradient for body size exists across the range of the possum in Australia (Tyndale-Biscoe 1973), with possums from the southern latitudes of Tasmania being larger than their more northern counterparts. It is possible that a latitudinal or an altitudinal gradient for size exists within the State. Individual mature males classified as mature females may come from more northern populations or from lower altitudes where sexual maturity is attained at a smaller size than in more southern or higher altitude populations. The males with small testis weights that were classified as immature may be from populations further south and/or from higher latitudes where maturity occurs at a higher body weight. If this is correct these males would be classified as immature individuals. This hypothesis requires further investigation. It was not possible, however, to examine the hypothesis in this study due to the small sample size.

Another explanation for the apparent misclassification is related to Dunnet's (1964) and Winter's (1977) observations that there are two types of male possums —dominant, usually older, resident males that occupy a stable, discrete home range, and subordinate, often immature, transient males that do not possess a definite area. Winter (1977) also noted that young males in the process of establishing themselves sometimes occupied areas that completely overlapped with an older, established male. In this situation a dominant-subordinate relationship existed with the older males scent marking exclusive areas such as dens trees, as well as being more likely to mate with females in the area. The status of the individuals collected in this study is not known, but it is probable that subordinate and dominant males were collected. Experiments conducted by Biggins (1979) revealed that dominant individual scent mark with the sternal gland more often than subordinate individuals. Greater rates of marking, larger scent glands and more secretion have been recorded in dominant individuals of many species including, lemurs *(Lemur catta)* (Kappeler 1990), bank voles *(Clethrionomys glareolus)* (Gustafsson *et al* 1980), guinea pigs *(Cavia porcellus)* (Beauchamp 1974) and rabbits *(Oryctolagus cuniculus)* (Mykytowycz & Dudzinski 1966). It is also well known that size is an important factor in dominance relationships (Francis 1988), with dominant males usually being larger (eg golden hamsters, *Mesocricetus auratus* (Drickamer *et al* 1973), eastern chipmunks, *Tamias striatus* (Brenner *et al* 1978) and having higher levels of testosterone than subordinate conspecifics (eg rhesus monkeys (Rose *et al* 1971) and reindeers, *Rangifer tarandrus* (Stokkan *et al* 1980)). It is possible that the larger mature males correctly classified are older, dominant individuals that have an established home ranges. The smaller mature males that were classified as mature females may be transient individuals that have reached sexual maturity but do not have a defined home range, or younger males whose home range overlapped completely with an older, established male. And the mature males classified as immature may really be immature males (based on testis weight) that are approaching maturity. These individuals may or may not have left the maternal home range.

Misclassification of mature females was very high (almost 70%). Differences in classification may be related to the reproductive state of the females. Bolliger and Hardy (1944) observed that the sternal fur in female possums usually starts appearing moist when the pouch young is 2-3 months old. Similarly, Winter's (1977) observed that the majority of scent marking by females using the sternal gland occurred between the first and last time the joey was seen riding on the mother's back. In this study (see Chapter 5 for details) the

rate of sternal marking in females carrying pouch young was higher than in oestrus or anoestrus females; no data on the rate of marking in females with young on their back was collected. (Possums spend their first 4-5 months in the pouch and another 1-2 riding on her back (Dunnet 1956; How 1983)). Observations of oestrus females engaged in consort behaviour with males reveals that females are more tolerant and less aggressive towards males during this time (Winter 1977). The rate of sternal marking in oestrus females in this study (see Chapter 5) was low. Based on these observations it might be expected that females with pouch young or young on their backs might show greater sternal gland development than females in oestrus or anoestrus. The results, however, do not support this theory. Females with pouch young or evidence of pouch young or dependent young were found in all three classifications, ie mature females correctly classified, mature females classified as mature males, and mature females classified as immature. Although more than half of the mature females with the greatest tissue development (ie those classified as mature males) had pouch young or evidence of older dependent young (and might therefore be expected to have higher levels of marking), anoestrus and oestrus females were also found in this group. In the group of mature females classified as immature, individuals in all reproductive states were collected. Among the "correctly" classified mature female the majority had pouch young or evidence of dependent young; one female could be positively classified as being in oestrus.

There are two issues that may be confounding the data and causing the apparent misclassification. As outlined in the methods, there was some difficulty in classifying the reproductive state of individual roadkill females. Some misclassification at the histological level is probably due to misclassification of the reproductive state. The degree of apparent misclassification may have been lower if it had been possible to accurately divide females into groups based on the presence or absence of young and on the age of the young. It is unlikely, however, that improved classification of the reproductive state would have separated the females on the basis of reproductive status.

The second confounder is the possibility of a habitat and/or a latitudinal or an altitudinal gradient for body size, as discussed above. The relative size at which females become reproductively mature may differ depending upon their location in the State. Although not significant, there was a trend for mature females classified as immature to be smaller and mature females classified as mature males to be larger than the correctly classified mature females. The lack of significance in the trend may be due to the small sample size.

Another possibility is that classification of mature females with respect to sternal gland development may have less to do with their reproductive state and may be more associated with resource holding and protection. The majority of mature females in a population breed each year (Bolliger 1940; Dunnet 1964; How 1983); individual access to resources, such as den sites, however, differs (Winter 1977). Female possums do not necessarily have home ranges that are exclusive to other females (Dunnet 1956, 1964; Crawley 1973; Winter 1977) and younger females may share an area with an older female, usually their mother (Winter 1977). Winter observed, however, that like males, female home ranges show an age-structure pattern and dominance hierarchies correlated with age and/or size exist. Older females have exclusive areas in their home range (den trees and food trees) relative to other members of the same sex and dominance status; scent marking is concentrated at these sites. Younger females are able to share parts of the home range as long as they avoid the more dominant female. The variation in sternal gland development among mature females may be related to their status in the population. The females classified as mature males may be older more dominant individuals with established ranges and adequate resources. The other mature females may be younger, subordinate individuals that occupy areas with fewer resources.

This theory does not exclude the observation that marking occurs more frequently in females with young. Older, more dominant individuals that have larger glands and higher rates of scent marking may have a selective advantage over younger subordinate females. Increased marking of resources such as food tree and den sites may enhance the survival of

the young. Marking of food trees may be related to the increased nutritional requirements of females with young and/or the possible decrease in food availability during the winter months when the majority of the young are developing in the pouch. Marking of den sites may be associated with an increased need for an adequate daytime shelter during the harsher winter months. Although the majority of females in a population breed each year (Bolliger 1940; Dunnet 1964; How 1983), in the field study described in Chapters 4 & 5 the number of females successfully rearing young was low.

2.4.2. Gender by season

Among mature possums a number of significant seasonal differences in the histology of the sternal gland exist between the sexes and within each sex. In the previous section significant differences between mature males and females were found, with males having greater glandular tissue depths and a higher percentage of each tissue type. Including season in the analysis reveals that mature males do not have significantly more glandular tissue than females at all times of the year. In considering differences between the sexes it is most useful to compare males and females at the same time of the year (ie the first row of Table 13). The greatest differences between the sexes are seen during the breeding, post breeding and dispersion periods. All the significant differences between males and females are seen in the total depth of the glandular tissue and in the holocrine sebaceous tissue parameters; there were no significant differences in any of the specific sudoriferous apocrine tissue parameters. No significant differences between the sexes were observed in the pre-breeding season. Reasons for differences in the sternal gland histology of the sexes across the seasons are discussed below.

For mature males, differences in sternal gland histology are closely related to the breeding season. Comparison of the sexes during the breeding season revealed that males have significantly greater total tissue depth and holocrine sebaceous tissue than females. Analysis of seasonal differences among the mature males only showed that the size of the holocrine sebaceous nuclei was the most important parameter for separating males by season. The nuclei were found to be significantly larger in the pre-breeding and breeding seasons than in the post-breeding period. In the breeding season and the period leading up to breeding, therefore, holocrine sebaceous glandular tissue development and activity are at their greatest.

These findings correlate well with what is known about olfactory communication and the ecology of male possums. Scent marking in males is known to increase during the breeding season. Winter (1977) recorded peaks in sternal gland marking in March and September, which correlated with peaks in conception. A large percentage of this marking occurred in the presence of females in oestrus. During breeding times males often form non-permanent consort relationships with females for a period before mating takes place (Winter 1977). Salamon (1998) found that secretions from the sternal gland of free-living male possums contained the greatest number of compounds during the main breeding season between April and May. In September of one year and October of another the number of compounds in male secretions also showed a peak, though not as high as in April and May. This corresponds with the secondary spring breeding season seen in some populations. The prostate gland of males increases in size from March to May (Gilmore 1969) and levels of testosterone show a peak in March (Gemmel *et al* 1986). Testosterone has been shown to positively influence the size of the sternal gland (Bolliger 1944a & b; Biggins 1979). Gemmel *et al* (1986) reported that testosterone levels are at their lowest in September; it is not clear whether the animals examined came from a population that experience a secondary peak in breeding at this time.

Peaks in testosterone levels, increases in scent gland size, secretion and associated scent marking behaviour leading up to and during the breeding season are common among

mammalian males. When female camels *(Camelus dromedarius)* are in oestrus, androgen levels peak and secretions from the neck gland increase in males (Yagil & Etzion 1980). Enlargement of the temporal gland and production of copious secretions occurs during the period of musth in African elephants *(Loxodonta africana);* testosterone levels are also higher during at this time (Poole *et al* 1984).

Examination of the seasonal differences between the sexes also shows a significant difference between males and females during the dispersal period, with males having greater holocrine sebaceous tissue depth. This difference is due, at least in part, to the low holocrine sebaceous tissue depth among mature females at this time of the year, rather than particularly high levels in males. Analysis of males alone does not indicate that development of the holocrine sebaceous tissue is particularly increased at this time of the year. Indeed, the percentage of holocrine sebaceous tissue is at its lowest, and the depth of holocrine sebaceous tissue and the size of the nuclei are also low. Furthermore, Winter (1977) noted that sternal gland scent marking by mature males was very low between October and December.

Dispersal is a time of major change for both adult and juvenile possums, and it would be expected that olfactory communication would have a role to play. During this period juveniles born in the previous autumn breeding season become independent from their mothers and may move out the maternal home range. Juvenile males and females show different patterns of dispersion: males tend to disperse further from the maternal home range than females and females are more likely to establish home ranges within or adjacent to the maternal home range (Dunnet 1964; Winter 1977; Ward 1985). These differences may be due to the observation of Dunnet (1964) that young males are tolerated by other males until they begin to mature, at which time "they are apparently driven out". Dunnet does not suggest any mechanism that mature resident males might use to move juvenile males out of their home range. Winter (1977) suggested it was probable "that adult male aggression is responsible, partly if not wholly, for juvenile dispersal". Although he observed few aggressive encounters between resident adult males and juvenile males born within their home range, almost all the encounters between adult males and young males of unknown origin were of an aggressive nature.

Given the well-established associations between aggression, testosterone and dominance relationships among mammals, and the knowledge that testosterone influences development of the sternal gland in the possums, it is reasonable to hypothesise that increased aggression in mature males toward dispersing juvenile males might be reflected in the histology of the sternal gland. Although development of holocrine sebaceous component of the sternal gland and the level of scent marking by mature males is low between October and December, analysis of chemicals from sternal gland secretions present a different picture. Examination of secretions in one captive adult male by Salamon (1998) revealed that 50% of the compounds measured in sternal gland secretions showed their highest concentrations in November. The highest concentrations of nonanal, a chemical found in secretions at all times of the year, was also found in November in all animals examined. Histological data collected in this study indicates that many of the sudoriferous apocrine tissue parameters in mature males show the greatest amount of development during the dispersion period. If it were possible to identify the status of mature males in the roadkill sample, the results might be expected to show that more dominant males with established home ranges have better developed apocrine tissue during the dispersion period than more subordinate, non-range holding individuals.

Sternal gland scent marking in mature males is associated predominantly with the breeding season. Examination of the histology of the sternal gland indicates that that sternal gland scent marking in mature males corresponds to the greatest development of the holocrine sebaceous tissue components of the sternal gland. Secretions deposited during sternal rubbing during the breeding season, therefore, probably contain a large percentage of material produced by the holocrine sebaceous components of the sternal tissue. Conversely, sternal gland scent marking and holocrine sebaceous tissue development

during the dispersal period are lower. Sudoriferous apocrine tissue development, however, shows some evidence of being greater in mature males at this time. Secretions probably contain a higher percentage of material produced by the sudoriferous apocrine tissue at this time of the year. Not all odorous secretions are deposited in the environment in the form of a scent mark, scent can be released directly into the air (von Holst 1985). It is possible that odours in secretions from the sternal gland are perceived by dispersing juvenile males during aggressive interactions with mature, home range holding males. In this type of close interaction scent marking the substrate is probably not necessary for two reasons. Firstly, if the odour is volatile and evaporates from the surface of the skin and stained hair, the closeness of the interaction may make scent marking redundant. Secondly, scent marking of objects in the environment may be regarded as a method of communicating a long-term message, one that will function after the marker has left the vicinity. In aggressive interactions the visual and auditory behaviour of the individuals conveys an immediate message, and any olfactory communication at this time needs to also be immediate. It should be noted, that under experimental conditions Biggins (1979) observed sternal marking in dominant males in agonistic encounters between males. It is difficult to relate this observation to what occurs under natural conditions as these captive animals were not in situations analogous to those that might be experienced in the field during the dispersal period.

Differences in the types of interactions engaged in by mature male possums at different times of the year may explain why the sternal gland is composed of two types of glandular tissue and why the maximal development of each type occurs at different times of the year. During the breeding season males spend their time accompanying oestrus females (Winter 1977). This consort behaviour functions to increases the consort males chance of mating with the female and also serves to reduce the level of aggression of the female when mating takes place (Winter 1977). By accompanying the female a male is able to prevent other males from approaching her. An increase in scent marks around the home range of the male may inform other males of the resident's occupation of the area. Previous experience may deter adjacent individuals of a similar or lower dominance status from pursuing any female residing in the same area. By scent marking the male is advertising his presence which may in turn decrease the chance of aggressive encounters with other males. If a male has to continually engage in encounters with other males he may miss the opportunity to mate with the female and another male may mate with her while he is occupied. For a scent mark to function for a period of time after deposition it must persist in the environment. The holocrine secretion from sebaceous glands, sebum, is oily and rich in lipids (Flood 1985) and has a viscous or semi-solid consistency (Strauss & Ebling 1970). Regnier and Goodwin (1977) demonstrated that sebum retards the evaporation of phenylacetic acid, a behaviourally active component of secretion from the sebaceous midventral gland of the Mongolian gerbil *(Meriones unguiculatus)* (Thiessen *et al* 1974). Sudoriferous apocrine secretions are watery, rather than oily (Flood 1985). This characteristic may enable odorous compounds to readily evaporate into the air upon reaching the surface of the skin. Secretions from holocrine sebaceous tissue may persist in the environment and function over a period of time, whereas secretions from sudoriferous apocrine tissue may function in the short term.

Biggins (1979) and Salamon (1998) have shown that the range and amounts of polar and non-polar, volatile and highly volatile and less volatile substances vary between individuals, between the sexes, between animals of different age and status, and between seasons. Among captive possums, Biggins (1979) found that secretions from the sternal gland of a socially stressed male did not contain several of the more volatile compounds found in other males. This provides some support for the hypothesis that volatile odours may be present in secretions of dominant males during the period of dispersion. If juvenile males are able to detect odours from mature males during agonistic encounters they may later recognise and associate odours deposited as scent marks by the same male with aggressive interactions and avoid areas marked by the resident male.

Analysis of the histology of the sternal gland reveals that, like mature males, the size of the holocrine sebaceous nuclei shows significant differences between the seasons in mature female possums. The nuclei were significantly larger during the pre-breeding period compared than the post-breeding and dispersion periods. Examination of the descriptive data reveals that all the holocrine sebaceous tissue parameters showed the greatest amount of development during the pre-breeding period. No clear trends in the sudoriferous apocrine tissue are apparent.

It is difficult to interpret the histological findings in the context of what is known about the biology and olfactory communication in the mature female brushtail possum. As mentioned earlier Winter (1977) reported that females mostly scent mark between the period when the young first leaves the pouch and the last time they are seen riding on the mother's back. And Bolliger and Hardy (1944) observed that the sternal fur in female possums usually starts appearing moist when the pouch young is 2-3 months old. Seasonal differences in sternal gland secretions have been found in females (Salamon 1998) but no information on the reproductive state was given.

As described in the discussion of gender and maturity, the sample of animals used in this study have some inherent problems. There may be differences in the timing of the seasons in different areas of the State which make it difficult to draw out significant seasonal changes in the histology of the sternal gland. Although most young are born in the autumnal breeding season, some populations have a smaller secondary breeding season in spring, and births have been reported for all months of the year. Even within the main breeding season there is variation in the timing of births across the range of the possum. In Australia around Sydney (Bolliger 1940) and Canberra (Dunnet 1956, 1964) autumn breeding from March to April and from March to May, respectively, have been reported. In Adelaide breeding may start as early as January (Pilton & Sharman 1962), while in Tasmania is may not start until April (Lyne & Verhagen 1957). In New Zealand, where the possum was introduced in the nineteenth century (Wodzicki 1950; Pracy 1962), breeding as been reported from March to June (Tyndale-Biscoe 1955), March to May (Gilmore 1969) and April to June (Crawley 1973). On a smaller scale variations in the timing of breeding within populations from year to year have been reported (conference presentation by Brockie *et al* cited by Hocking 1981). And variations between adjacent populations occupying different habitats have been observed (Hocking 1981). The geographical spread of possums collected in this study may be a factor in the variation in the timing of breeding in this study, which can be seen in the range of reproductive states of mature females in each season. In this study females with young were collected during all four seasons. Therefore, if changes in the histology of the sternal gland are related to reproduction in the female analysis by season is not the best method to use for females. The next section examines the results of female classified on the basis of their reproductive state.

2.4.3. Females by reproductive state

To determine more clearly any changes in the histology of the sternal gland with reproductive changes, females of different reproductive states were examined. As expected, in the analysis that included immature females, the sternal gland of immature females differed significantly from all three mature female groups. All three depth parameters were significantly less in immature females, as they were in the earlier analysis of females by maturity. In this analysis separation of the mature females in the canonical variate plot is greatly influenced by the large difference between immature and mature females. To eliminate the influence of the immature females on the analysis the three mature female groups were examined without the immature females

Examination of mature females only revealed no significant differences in the histology of the sternal gland between mature females of different reproductive state. There are a number of reasons why no significant differences were found. Firstly, the sample size is

very small. It is possible that there are differences that cannot be detected when the number of individuals is low. Examination of the descriptive statistics for each tissue parameters provides some evidence that the sternal gland does alter with female reproductive condition. For example, among the oestrus females holocrine sebaceous tissue depth was at its lowest and holocrine sebaceous nuclear diameters at their smallest. This corresponds with a period of decreased female aggression towards males (Winter 1977) and the observation in this study (see *Chapter 5*) that the level of sternal gland scent marking in oestrus females is low compared to females with young. While holocrine sebaceous tissue parameters are low in oestrus females, sudoriferous apocrine tissue depth shows its greatest level of development among this group. The greatest development of holocrine sebaceous tissue is seen in female in anoestrus condition.

Difficulties, as outlined in the methods, in classifying females by reproductive state may also influence the results. Misclassification may be masking significant differences between the reproductive states. The observations by Bolliger and Hardy (1944) that the sternal gland in female become moist when pouch young are a few months old and by Winter (1977) that females mark more often when they have a young on their back, suggests that if it had been possible to separate females with small pouch young and females with dependent young on their back, rather than grouping them, together significant differences may have been found.

The results of this study indicate that further examination of the histology of the sternal gland is required. Errors and potential confounders need to be identified and taken into account. A method that eliminates the problem of misclassification of the reproductive state of females should be used. Potential differences in the relative size of animals attaining sexual maturity resulting from the collection of animals over large geographical areas also needs to be addressed. Attempts were made to overcome both these issues in a field study (see Chapters 4 and 5). Using the tissue core sampling method of Bradley and Stoddart (1991m/s) small samples of sternal tissue were collected from live animals from a known population. Unfortunately, although the method was used successfully in the field it was abandoned after a few months. This was because collection of the sternal tissue made it difficult to collect information on sternal gland scent marking, which was a main aim of the field study. Location of sternal scent marks was determined using a combination of spool-and-line tracking and fluorescent pigments applied to the sternal region. To eliminate any possibility of infection, fluorescent pigment was not applied to the sternum of individuals immediately following collection of sternal gland which left a small wound (approx 3mm in diameter). This meant it was not possible to assess the status of the sternal integument tissue parameters while simultaneously measuring the amount of scent marking. Continued collection of sternal gland samples would have greatly decreased the amount of information that could be collected on scent marking.

Collection of histological material by core sampling in a population of known individuals has advantages over collection of material from roadkills. Firstly, all the animals are from the same population and this eliminates possible confounding due to relative size differences in animals of similar status from different areas. Secondly, the status of individuals can be ascertained — the reproductive state and dominant-subordinate/resident-transient status. Thirdly, changes in histological parameters of an individual animal can be monitored over time —changes as an individual matures, changes through various reproductive phases, and changes from season to season.

Despite the difficulties, a number of differences between different classes of possums are evident from this preliminary investigation of the histology of the sternal gland. There are no significant differences between immature possums of either sex. Immature and mature animals differ in the development of the sternal gland: mature animals have greater amounts of both glandular tissue types. The total depth of glandular tissue separates immature and mature males and immature and mature females. Seasonal differences between mature males and females are related to the respective activities of each sex at a particular time of the year. Mature males show the greatest level of holocrine sebaceous

tissue development during the breeding season; and sudoriferous apocrine tissue is best developed during the dispersion phase. Among mature females there are some trends in tissue development related to the reproductive state of individuals.

The significant differences and non-significant trends in this study indicate that differences in the histology of the sternal gland exist between different groups of possums. The analysis used highlights common aspects of the histology within groups that separate them from other groups. The common histology within groups probably leads to the production of odours that are specific to and recognisable as belonging to a particular group of possums, ie mature males, oestrus females etc. Individual differences in the histology of the sternal gland, especially between individuals within a gender, age, and reproductive groups, were not examined in this study. Such differences, however, may result in the production of chemical compounds that give an individual a distinct odour profile that may enable individual identification to occur.

Chapter 3. Recording Scent Marking in the Field

3.1. Introduction

Observing scent marking by mammals in the field is laborious, particularly when the species under observation is nocturnal, cryptic, partly arboreal, and covers large distances. As part of a study investigating the sternal gland of the brushtail possum *(Trichosurus vulpecula)* a method for recording sternal gland scent marking in the field was needed. The sternal gland of the possum is visible as an area of stained fur along the sternum (Bolliger 1944a & b; Bolliger & Hardy 1944; Green 1963). The region produces moist, frequently copious secretions that are deposited on the substrate in a forward rubbing movement known as "chesting" or "sternal rubbing". The possum adopts a bent leg stance and lifts its chin away from the substrate, which enables the chest to come in contact with the substrate. In a forward motion the chest is rubbed up the substrate and then lifted off (Winter 1977). Chesting may be a single rub of short duration; a single, more deliberate, longer duration rub; or a repeated movement.

Direct observation of sternal gland scent marking in brushtail possums is difficult. Because the species is nocturnal a source of artificial lighting or night vision equipment is needed to view the animal. Use of red lighting techniques was attempted (see *§ 3.2.3 Direct observation using red spotlights*), but its application was limited. The greatest problem with this technique is disruption of the normal behaviour of the possum. Although Winter (1977) successfully observed brushtail possums in field conditions using spotlights he noted that animals sometimes reacted to having the light shone on them. Other factors that make it difficult to observe possums in their natural state without disrupting their behaviour include the species' semi-arboreal habits and cryptic nature. These things, combined with the large distance covered by individuals during a night (eg males 572.5m ± 279.3, females 234.3m ± 173.0, Winter 1977), make it impossible to observe them from one vantage point or to maintain constant visual contact in even the most open habitats. Any movement or noise made by observers in an attempt to follow an individual results in the animal scurrying into a den opening or other refuge where they can remain for the rest of the night.

There have been very few studies of scent marking in nocturnal species under natural conditions. Review of the literature reveals that most studies of scent marking have been direct observational studies done in laboratories or under semi-natural conditions in outdoor enclosures using variations of the focal animal sampling techniques developed by Altman (1974). Direct observation is a useful technique when it is possible to observe the animal without disrupting its normal behaviour. The advantages of being able to observe animals directly include gaining information on the context, timing and location of scent marking.

Due to the difficulties in obtaining information on scent marking in possums directly a review of remote monitoring techniques was undertaken. Although remote monitoring may lead to a loss of information on the location, timing and/or context of the scent marking behaviour, it overcomes the problems of behaviour disruption experienced using direct observation in this species.

What follows is an examination of the techniques trialed and tested in an attempt to find a suitable method for recording scent marking in the brushtail possum in the field. The advantages and limitations of each technique will be discussed.

3.2. Methods and Discussion

3.2.1. Acoustic bio-telemetry

Acoustic bio-telemetry systems have been used successfully in a number of species to remotely monitor a range of behaviours. Sounds of feeding, drinking, sniffing, walking, threat huffs, moving in dense vegetation and digging have been recorded using collar-mounted transmitters in captive and leash-led Indian crested porcupines *(Hystix indica)* (Alkon & Cohen 1986; Alkon *et al* 1989). Jenness and Greager (1976) and Greager *et al* (1979) successfully used a collar-mounted acoustic transmitter to distinguish between feeding, grooming, calling, general activity and inactivity in free-living brush-tail possums.

The advantages of these remote monitoring systems include the following. Firstly, it makes it possible to obtain information on the activities of animals that are difficult to observe directly. Secondly, it is possible to have a system that records the information for replaying at a later date, thus eliminating the need for the observer to be present at the time of the activity. This also means that more than one animal can be "observed" at one time — something that is difficult to do directly without more than one observer, or without switching attention between animals. Thirdly, there is a range of sound analysis/signal processing packages that can be configured to automatically classify different sounds. Such technologies enable large volumes of data to be processed comparatively quickly, thus freeing the researcher from an otherwise tedious, time consuming task.

In the present study it was hypothesised that it may be possible to distinguish the sound of sternal gland of the brush-tail possum being rubbed against objects in the environment. Extensive testing of a collar-mounted acoustic transmitter produced by *Sirtrack Electronics* (DSIR, Havelock North, New Zealand) on captive possums revealed that the action of the possum pressing and rubbing its sternal gland on a variety of objects did indeed produce a sound that could be distinguished from the other behaviours recorded in possums previously by Jenness & Greager (1976) and Greager, Jenness & Ward (1979). Unfortunately, it was not possible to discriminate between the sound of marking actions and other movements made by the animal. This was due to a number of limitations of the devices being used. Firstly, the acoustic transmitter was a collar-based unit. Although small enough to not hinder the marking action of the possum, when the possum did mark the sound recorded was accentuated by the scrapping of the transmitter against the substrate being marked. This in itself was not a problem as it produced a very distinctive, easily distinguished sound. The problem was that the transmitter was often knocked and scrapped on objects as the possum moved around, particularly as it emerged and returned to its nest box and climbed around dense vegetation within its enclosure. The sounds made by such actions were very difficult to distinguish from the sound resulting from deliberate scent marking activities. Similar situations would have occurred in a field situation resulting in a gross over estimation of the frequency of scent marking in the species. Secondly, any differences between the sound of marking activities and the "accidental" sounds could not be audibly distinguished, and suitable computer software to aid in discriminating between different sounds was not available at the time the research was undertaken. Although it was not possible to employ remote acoustic monitoring as a method for recording scent marking activities in this study it is probable that with improved

technology the use of acoustic transmitters for the detection of scent marking activities may be a very useful technique. The development of software with the capacity to distinguish between a range of similar sounds may make it possible to separate not only sternal gland scent marking from other sounds, but distinguish between the various patterns of sternal gland marking (ie the short, lightly pressing chesting movements, the long, more deliberate chesting movements, and the combined chesting and chin-wiping movements, all of which may be single or repeated actions) and even marking of different objects within the environment (for example, tree trunks, rocks, grasses etc).

3.2.2. Implantable transmitter

Development of an implantable transmitter to remotely monitor scent marking was begun in the School of Zoology at the University of Tasmania. The device consisted of a transmitter connected to an electronic button, which was to be positioned under the sternal integument of the possum below the clavicles. When a possum scent marked by pressing and rubbing its sternum on the substrate the button would depress triggering an electronic signal that was picked up by an automated receiver.

This remote monitoring system had a number of advantages. Firstly, the identification of the individual marking is known. By using a number of transmitters and receivers it would be possible to record marking behaviour in a number of individuals at the same time. Use of a timing devices would enable the exact time of the scent marking behaviour to be recorded, making it possible to discern any temporal patterns in nightly marking behaviour. And, information could be collected over extended periods of time without requiring an observer to be present.

Despite the advantages, development of the technique was abandoned before trialing of the system in captivity began due to serious limitations. Animals would have to be removed from the field to conduct the surgical procedure necessary to implant the transmitter. There are a number of problems associated with capturing the animal, transporting it back to the laboratory, conducting the surgery, allowing recovery time from the anaesthetic and for wound healing, time to test the device in captivity and then release back to the environment. Firstly removal of the animal, even if sedated, and the other procedures would be highly stressful for the animal. Secondly, removal from the habitat may result in another individual "claiming" the home range of the animal that has been removed. These problems would be encountered again when the animal was recaptured to enable the device to be removed. This disruption would cause an unacceptably high level of change to the dynamics of the population under observation thus defeating the purpose of observing the study animals in the natural environment.

3.2.3. Direct observation using red spotlights

As well as the remote monitoring techniques discussed above, one direct observation method was trialed. Winter (1977) successfully used spotlighting techniques to observe scent marking behaviour in brushtail possums under field conditions. Continuous observations of possums were made possible by a number of factors. Firstly, the study was conducted in habitat that was originally shrubby open eucalypt forest, which had been modified by cattle grazing to form a grassy open forest. The canopy of eucalyptus trees was open making it easy to view the possums, the crowns of the trees were non-contiguous which meant that possums had to come down to the ground in order to move from one tree to another, and the grassy ground cover was short due to cattle grazing.

Observation of scent marking by direct observation using red lights was used under field conditions in this study. Observations were made as part of an investigation of den usage by possums. Between September and December 1994 four possums (2 males and 2

females) were fitted with collar-style radio-transmitters. During the day the den site of the animal was located using a handheld Yagi antenna and receiver. One animal was selected each night for observation. Observations of the possum emerging form its den site were made using a variable intensity spotlight fitted with a red cellophane filter. While it was possible to observe possums while they remained in the den tree, observation was impossible once they moved into another tree or down to the ground for a number of reasons. Firstly, although the field site selected was a relatively low (~ 30m) and open dry sclerophyll forest, the canopy was dense enough in places to make it difficult to observe possums moving through the canopy. Secondly, even though the ground cover was relatively open and consisted for the most part of low grasses and scrubs, once a possum was on the ground it was impossible to maintain visual contact even in the most open areas of the site. Attempts were made to follow individual animals but the slightest noise made by an observer moving through the scrub caused the animal to scatter or climb up the nearest tree where it would sit, apparently watching the observer, for hours.

Even under the "ideal" habitat conditions experienced by Winter, spotlighting still has its limitations. In this study, as in Winter's, the spotlight was observed to disrupt the normal behaviour of the possum. For the first few nights of observation an individual appeared to be aware of the presence of the observer. Akin to Winter's experience, possums would return to the den when the spotlight was shone in their direction and not re-emerge for hours. During 1160 hours of observation over four years Winter was able to adjust his spotlighting technique to suit the idiosyncrasies of individual possums, and it is probable that some habituation to his presence occurred. In the present study individuals did habituate to the spotlight after three to four nights of successive observations. The habituation appeared to wane if an individual was not observed for a few days, however. As in Winter's study it was possible to reduce the disturbing effects of the spotlight by adjusting the intensity of the spotlight depending upon how far away the possum was, and by directing the spotlight to one side of the animal. Despite these precautions use of spotlight did at times effect the normal behaviour of the possum.

While spotlighting has a number of advantages over remote methods of recording scent marking, including information on the timing, location and context of the behaviour, it was not found to be a suitable method for the field site in this study. Even though the site was very open compared with much of the natural habitat occupied by possums, it was not possible to observe scent marking behaviour once an individual had left its daytime den tree. Even if it had been possible to observe possums as they travelled along the ground a huge amount of time would have been needed to establish and maintain habituation of individuals and to then collect sufficient data to make the study worthwhile. Unfortunately, most research projects do not have the same time resources that Winter had. To overcome these difficulties the techniques outlined in the following section were used to obtain information about sternal gland scent marking in the brushtail possum.

3.2.4. Spool-and-line tracking and fluorescent pigments

The method eventually used in the study involved a combination of spool-and-line tracking and application of fluorescent pigments.

The use of trailing threads to track animals has been used infrequently since the 1920s. Breder (1927) and Stickel (1950) used threads to study the activities of turtles *(Terrapene c. carolina)*. More recently spool-and-line devices have been used to track the movements of a variety of species, including Tasmanian devils (*Sarcophilus harrissi)*, spotted-tailed quolls (*Dasyurus maculatus)* and eastern quolls (*Dasyurus viverrinus)* (Jones 1995); the European badger *Meles meles* (Hawkins 1989); New Guinean dasyurids (*Murexia lungicaudata, Antechinus naso & A. habbema*) (Woolley 1989); the New Guinea spiny bandicoot, *Echymipera kaluba* (Anderson *et al*1988), New Guinea giant rats, *Mallomys rothschildi* (Berry *et al*1987); meadow voles *Microtus pennsylvanicus* (Boonstra & Craine 1986);

1986); armadillos *(Chaetophractus cillerosus)* (Greegor 1980); a range of Brazilian mammal species (including endetates, marsupials, rodents and carnivores) (Miles, de Souza & Povoa 1981) and; three marsupial species *(Petaurus breviceps, Antechinus stuartii* and *Cercartetus nanus)* (Carthew 1994). In these studies spool-and-line devices have been used to locate nest and refuge sites, obtain information on home range and distances traveled, and study the habitat use, foraging behaviour and movements of individuals. The aim of this study was to assess the usefulness of spool-and-line devices as a tool for detecting scent marking in the brushtail possum *(Trichosurus vulpecula).*

The devices used in this study were based on the design of Jones (1995). Each device consisted of a series of 240m long centre-wound nylon spools (size 15, Penguin Threads Pty. Ltd., Victoria). Individual spools were wrapped in two layers — an inner layer of clinging plastic food wrap (*Glad Wrap*, Glad Products of Australia) and an outer layer of adhesive bandage (*Elastoplast*, Smith and Nephew (Aust.) Pty. Ltd.). The adhesive bandage was used as a foundation to attach an outer casing of adhesive bandage to hold a series of spools together; the cling wrap allowed the thread to feed out completely without becoming stuck to the adhesive bandage. A series of 3-5 spools were connected together by tying the end of one spool to the beginning of the next using a secure knot. (The number of spools used depended upon the length of thread required; i.e. 3 spools = 720m, 4 spools = 960m & 5 spools = 1200m.) A spot of quick drying glue (*Supa Glue*, Selleys Chemical Company Pty. Ltd. Australia or *PRISM 454 general purpose instant adhesive glue*, Loctite Australia Pty. Ltd.) was applied to each knot for added strength. The series of spools were then held together by a casing of *Elastoplast* on three sides so that the knotted ends were left exposed. To aid in the collection of the spool line the next day, the outside of individual spools were coloured differently using waterproof permanent markers (*Artline 70 High Performance Permanent Markers*, Shachihata Product, Japan) before construction. Marking the spools in this way did not completely dye the spool — the inside of the spool absorbed only a small amount of colour. The result of this uneven colouring was that as each spool fed out there was an increasing amount of colour on the thread. This made it possible to determine the direction the animal was moving in situations where the spool line had been broken at a number of points. To ensure that the complete spool was collected, the last 2-3cm of the end spool was coloured with ink that had not been used to colour any of the other spools in the device.

The completed spool weighed approximately 18.4g for a 3 spool, 24.4g for a four spool, and 30.6g for a 5 spool device. These weights were well below the recommended 5% of body weight (Amlaner & MacDonald 1980) as an artificial load for terrestrial mammals.

Fluorescent pigments have been used for a variety of purposes in ecological studies. Movement of insects has been studied by dusting them with powdered pigments (Taft & Agee 1962); tracking movements, determining home ranges, locating nests and burrows, and discriminating various kinds of physical contact interactions have all been studied by dusting small mammals (particularly rodents) with powdered pigments or attaching movement activated "dust capsules" to their bodies (Duplantier *et al* 1984; Leman & Freeman 1985; Dickman 1988; Mullican 1988; Goodyear 1989; Wapstra 1994). In this study fluorescent pigment was used in combination with a spool-and-line tracking device to locate the position of scent marks within the home range of brushtail possums.

A small amount (approximately 0.15g) of daylight fluorescent powdered pigment (Abel, Lemon Co Pty Ltd Chemicals Division) was mixed until combined with 2 tablespoons of *Vaseline* (Lever Rexona, Australia). Four colours were used — aurora pink E1, fire orange E4, lunar yellow E27, and signal green E8. The most easily seen were pink, orange and yellow. Green was more difficult to pick up when following the spool line as it was very similar in colour to the vegetation.

Possums were caught in Mascot live wire traps (30x30x60cm) baited with apple. Animals were transferred to a pre-weighed hessian bag and weighed before being sedated with a mixture of *Ketamine Injection* (ketamine hydrochloride, 100mg/ml, Parnell Laboratories (Aust) PTY. LTD.) and *Rompun* (xylazine hydrochloride, 20mg/ml, Bayer Australia LTD,) given intramuscularly (Wright 1983).

A spool-and -line device was attached between the shoulder blades of the animal using *Supa Glue*. To ensure that the device was held securely a patch of fur the size of the device was clipped to 3-5mm in length before gluing. The open, knotted ends of the spool faced posteriorly to allow the spool line to feed out behind the animal as it moved forward. Approximately a ¼ of a teaspoon of the fluorescent pigment-vaseline mixture was then applied to the stained area of the sternal integument of a sedated possum.

Following the securing of the spool-and-line tracking device and application of the pigment the animal was returned to the hessian sack and left to recover from the anaesthetic. The beginning of the first spool was tied to the trap were the animal was caught. At dusk the fully recovered animal would leave the bag and the spool would feed out behind it. Any sternal scent marking was left behind on the object marked as a coloured streak of pigment and vaseline. Plate 5 shows the high visibility of scent marks deposited the field using this method.

Once the spool line had completely fed out all that was left attached to the animal were the adhesive plaster and the cling wrap. The remains of the device would fall off approximately 3 days following application. The majority of the devices fell off inside the trap when the animal was next captured (usually 2 days after application of the spool).

Before using this technique in the field, observations of captive animals fitted with both spool-and-line devices and pigments were made to assess any behavioural changes. No changes in behaviour were noted. The animals seemed to be oblivious to both the spool-and-line device and the pigment.

Spool lines were followed within 3 days of being laid down. A record of the path of the spool was made by taking compass bearings each time the spool changed direction and pacing the distance of each directional segment. A variety of information was recorded — scent marks, trees visited (for scent marking, climbing (den and/or feeding)), trees passed within 0.5m of the spool line), and traps investigated. All spool thread was collected to avoid confusion with later spools.

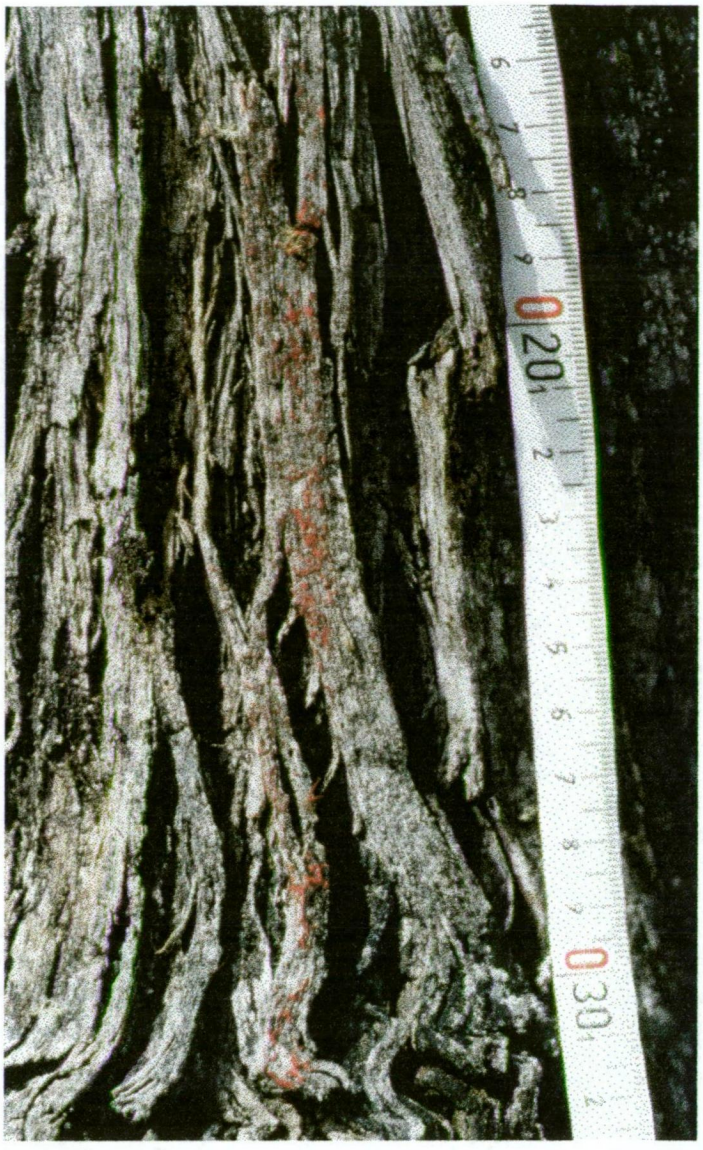

Plate 5. Sternal gland marking visible on tree trunk as a smear of red pigment and vaseline.
Scale: large divisions on tape measure = 1cm
small divisions = 1mm
(Photo: K. Hynes)

Sternal gland scent marking was recorded using these techniques between November 1992 and December 1994. A total of 106 spool-and-line observations were made (59 male and 47 female) using 14 individual animals (8 males and 6 females). Of these 99 were successful. On seven occasions (3 male and 4 female) the spool fell off while the animal was in the bag or within a few metres of leaving the bag. For most of the remaining spools information was collected for the complete length of the spool. There were a few situations that prevented data being obtained for the whole spool. Firstly, some animals went out of the study site (see *Chapter 4* for details). Even though the spool had worked, data from spool lines outside the study site could not be collected. Secondly, spools occasionally fell off the animals after they had left the bag. This usually happened when an animal was in a tree, particularly a den tree, or when moving through dense scrub/vegetation. Occasionally a spool was found on the ground and there was no apparent reason for it to have fallen off. The most likely explanation is that it was dislodged earlier in the evening when the animal was up a tree or in thick scrub, but did not fall off completely until later.

The combination of spool-and-line devices and fluorescent pigments was used successfully to study the position/location of sternal gland scent marks made by possums. As with the other remote monitoring techniques discussed, use of spool-and-line tracking and fluorescent pigments allows information on scent marking to be collected without an observer been present at the time of the marking activity. This is advantageous when the animal involved is nocturnal, and also means that the behaviour of the possum is not influenced by the presence of an observer. The techniques also allow for information to be collected on more than one animal at a time. One advantage of the spool-and-line/fluorescent pigment technique over the other remote monitoring techniques is that information on the location of scent marks and on the types of objects marked can be obtained. Such information usually requires direct observation of scent marking behaviour.

Spool-and-line tracking and fluorescent pigments have a number of other advantages. Fluorescent pigments not only allowed the location of scent marks in the habitat of the animal to be determined, but the size (length and width) of the scent mark could be determined. This may be an indication the type of sternal gland scent mark, that is, a single or repeated mark of short or long duration. Winter (1977) observed that different objects in the habitat were often marked using a particular variation of the chesting action. For example, logs were usually marked with a single quick rub, whereas marking on the ground or on tufts of grass was more thorough being a firm action that was repeated a number of times. Application of pigments to the captive animals may reveal that the different marking behaviours leave different patterns on the substrate being marked. Further more, by conducting observations under experimental conditions it may be possible to determine if the different types of marking action occur in different contexts (ie are due to different stimuli). If this were the case it would be possible to use the fluorescent pigment technique in the field to gain some insight into the context of the marking behaviour without having to make direct observations. Such an investigation was beyond the scope of this project but warrants further examination.

Fluorescent pigments also made it possible to obtain information on over-marking and remarking of scent marks. The pigment lasts for months in the environment when made on permanent surfaces such as smooth barked trees trunks, rocks etc. (Marks made on less permanent objects such as grasses, leaves etc last until the object decays or blows away). This makes it possible to see where a possum had marked on object marked previously by another individual or remarked where they had marked earlier.

Another advantage is the materials required are cheap and easy to construct. The spool-and line devices and pigment are easily applied and do not affect the behaviour of the animal. As well as being able to locate scent marks, additional information can be obtained on habitat use (trees, den sites, paths, etc), including the percent of distance spent in particular habitat types (eg in trees, on the ground, walking along fallen tree trunks, etc). Information on the location and size of home range, use of the home range and patterns of movement during different times of the year can be ascertained.

As with all techniques spool-and-line tracking and use of fluorescent pigments has some disadvantages and limitations. Firstly, following the spool line is very time consuming. For example, during winter, when daylight was limited, only one 4-spool device could usually be followed in a day. Secondly, it was not possible to easily record scent marking that had occurred high up on trees and inside den sites without using a ladder to climb up trees. Thirdly, the spools were often not long enough to last through the night — there were only a few occasions were the animal returned to a den site with spool still feeding out behind it. It is not easy to use longer spools as they are too bulky, rather than too heavy, for the animal to carry — the bulkier the device the more likely they are to scrape against objects in the environment and be knocked off. Another limitation was that it was not possible to use the spool-and-line device with female possums with young being carried on the back. Young on their mother's backs prevented the spool from feeding out cleanly and there was a possibility that the juvenile could become tangled in the line. Young following their mothers also encountered these problems. This meant that it was not possible to obtain information about the scent marking activities of females at this stage of the reproductive cycle. A major disadvantage, common to all remote monitoring methods, was that it was not possible to know the context in which the scent mark was made, ie. what may have motivated the animal to mark. Further more it was not possible to know the exact time that the scent mark was made, although the position of the scent mark along the spool line gave some indication of whether the mark was made early in the evening or later on.

Other difficulties included spools falling off, breaks in spool lines and line getting caught and dragged by other animals. These problems were relatively minor and for the most part could be overcome or decreased to some extent. Spools would fall off in the bag if the animal was not adequately sedated or started to come out of the anaesthetic while the spool was being attached, or if insufficient glue was used. Care was needed to ensure that the spool was securely attached and the glue had time to dry while the animal was still sedated. It was more difficult to attach the spool-and-line device when the animal was wet, although clipping the fur and removing any excess moisture with a towel eliminated this problem. Spools sometimes fell off when the animal moved through very dense vegetation or squeezed under solid objects (eg fallen tree trunks) causing their backs to scrap on the object resulting in the spool being rubbed off. This problem is difficult to overcome without positioning the spool-and-line device somewhere else on the animal. The device was placed between the shoulder blades as this location eliminated other problems such as the spool getting tangled around the possum as it fed out and the possum grooming to remove the device. Breaks in the spool line making it difficult to find and follow were a minor irritation, although colouring the individual spool lines helped overcome this. Occasionally a spool line would get caught and dragged by another animals. This was usually fairly obvious, however, as the path would change direction dramatically and then change back and continue in the original direction.

An important consideration is that pigment applied to the sternal integument lasts throughout the night and can last for more than one night, therefore it is important to use one colour pigment per animal in case the spool runs out and animal continues to mark during the night or on subsequent nights. Marks found can then be attributed to a particular animal if found at a later date, even if the specific date of marking is not known.

Despite the difficulties and limitation of the spool-and-line/fluorescent pigment technique these methods were used successfully to collect information on sternal gland scent marking in the brushtail possums. The advantages of the techniques make it suitable for use in other mammals, particularly nocturnal, wide-ranging, cryptic species, that are difficult to observe directly. Data collected using spool-and-line tracking and application of fluorescent pigments is presented in Chapters 4 and 5.

Chapter 4. Home Range and Den Sites

4.1. Introduction

The brushtail possum, *Trichosurus vulpecula,* inhabits most areas where there are trees, particularly woodlands and open forest. Although it is an arboreal species possessing sharp claws, an opposable first toe on the hindfoot and a prehensile tail, which it uses to climb, the brushtail possum spends much of its time on the ground. The species is generally solitary. The only long-term contact occurs between a female and her offspring (Tyndale-Biscoe 1973), with shorter periods of contact between adult males and females occurring during consort relationships prior to breeding (Winter 1977).

The brushtail possum is a nocturnal species that utilises hollows in tree branches and trunks, and fallen logs as a den sites in its natural habitat during the day (How 1983). Winter (1977) observed that den sites were important focal points in the home range of possums. Exclusive areas in home ranges existed among individuals of the same sex and status; such areas were concentrated around den trees. In interactions between adult females and males at den sites females were always dominant to males (Winter 1977).

Differences in the home ranges of possums exist between the sexes. Studies conducted in a variety of habitats in Australia and New Zealand have shown that within a particular habitat males generally have larger ranges than females (Dunnet 1956, 1964; How 1972; Jolly 1973 Crawley 1973; Winter 1977; Hocking 1981). The size of the home ranges varies greatly between the studies with reports for males ranging from 0.8 to 9ha and for females from 0.3 to 6ha (see *Chapter 1. Introduction* for more details).

As well as differences in home range size, the process of establishing a home range differs between the sexes. These differences are related to the patterns of dispersal of the young possums. Clout and Efford (1984) found that 59.1% of the males recruited into a population were of unknown origin, whereas 71.6% if the females recruited were from the local population. Juvenile males tend to disperse further from the maternal range than females (Dunnet 1964; Winter 1977; Ward 1985). This may be due in part to resident males excluding young males from occupied home ranges (Dunnet 1964). Dunnet observed that young males were tolerated in male ranges until they were about one year old. Once they begin to mature they were apparently driven out. Winter (1977) also proposed that adult male aggression was partly, if not wholly, responsible for the dispersal of juvenile males. Winter did not observe many aggressive encounters between resident adult males and juvenile males raised in the same area, but reported that most of the encounters between resident adult males and young males of unknown origins were aggressive in nature. Juvenile females are more likely to be recruited into the population (Clout & Efford 1984; Ward 1985), establishing home ranges within or adjacent to their maternal home range (Dunnet 1964; Winter 1977). Winter (1977) found that the dispersal of juvenile females did not appear to be influenced by adult males. The behaviour of an adult male towards a young female was neutral until she first came into oestrus and began to mature sexually. At this time adult males began to be sexually attracted to young females.

It has been suggested that there are probably two types of possums — those that are resident in an area and occupy a stable, discrete home range, and transient individuals (usually immature and male) that do not possess or occupy a definite area (Dunnet 1964). As well as young dispersing males, Dunnet observed transient adult males moving through

study areas. He concluded that these individuals were searching for vacant territories and reported several cases of adults settling in unoccupied sites.

Following observations of resident males, Dunnet (1964) concluded that males usually occupy mutually exclusive areas that are generally defended and could therefore be regarded as territories. In some areas Dunnet reported that a dominant and subordinate relationship existed between adult males who were not mutually exclusive. More recent studies do not concur with Dunnet's first observation. Crawley (1973) found extensive overlap between male ranges and did not observe any conspicuous territorial behaviour. A closer examination of Dunnet's data by How (1972) revealed that male ranges were not mutually exclusive and that nearly all overlapped with other males. In another study, Winter (1977) found that the home ranges of males did not generally overlap. Further more, he did not consider males to be completely territorial. The only evidence of territorial behaviour was observed at den sites, and here females were always dominant to males. Beyond den sites no evidence of patrolling or scent marking of boundaries was found (Winter 1977). Winter recognised two classes of males: younger adult males who do not have established home ranges and older established males. The older males were usually four years old or greater and were dominant to the younger males. These males appeared to have established home ranges that were stable. The ranges tended to have a core area that overlapped little with the core area of other adjacent established males. The younger males had home ranges that may have overlapped extensively with older males, but not much with each other. Winter considered that the younger males were in the process of establishing themselves. He observed that they tended to shift the centre of their activities over time and could disappear from an area altogether. Success in establishing a range that overlaps with an older male appeared to be related to being able to find an unoccupied den.

Among females, home ranges generally overlap to a greater or lesser degree (Dunnet 1956, 1964; Crawley 1973; Winter 1977) and may overlap completely with other females (Dunnet 1956, 1964). In areas where dens were well spaced the home ranges of females did not overlap (Winter 1977). As with males Winter (1977) observed dominance hierarchies correlated with age among female's home ranges. The home range of younger females (up to ~ 2 years of age) may overlap with the home range of older females, usually their mother, but not with other young females. As a female becomes older her range becomes more exclusive in relation to other older, established females. Females usually have established home ranges that are exclusive to other established females by the end of their third year. Within the ranges of both males and female intrasexually exclusive areas existed, usually in the immediate vicinity of the den tree (Winter 1977).

As well as overlap within the sexes, the home ranges of individuals of different sexes have been shown to overlap (Dunnet 1964; Winter 1977). Often one to two females overlap with a male range, although reports of up to eleven females overlapping with one male have been reported (Dunnet 1964).

Brushtail possums that possess home ranges are considered to be fairly sedentary (Dunnet 1964). There is evidence, however, that they make occasional sallies out of their usual home range (Dunnet 1956; How 1972; Winter 1977). Winter (1977) observed that males following oestrus females would venture out of their normal home range.

In this chapter and the one that follows (*Chapter 5*) the results a two-year field study conducted to examine sternal gland scent marking behaviour in the brushtail possum are discussed. The aim of the current chapter is to provide background information on the possums investigated in the field study to set the scene for the discussion of scent marking that follows in Chapter 5. This chapter focuses on the home range and den sites of possums captured during the study.

4.2. Methods

4.2.1. Study Site

The field study was conducted in the Mount Morrison State Forest near Nugent on the East Coast of Tasmania (see Figure 9 and Plates 6 and 7). (Universal Grid Reference 55GEN511737; Longitude 147° 37', Latitude 42° 44'). Forestry activities, including selective saw-log logging and woodchip logging, have been conducted in parts of the State Forest since the mid-1800's. From the 1850's until the early 1980's the region was subject to selective logging of saw logs. From the early 1980's to the present extensive logging for woodchips has been conducted. The study site was an area of open dry-sclerophyll forest located in the centre of the State Forest. It was a relatively undisturbed area of approximately 25 hectares. The early history of the site is unclear, although it is probable that large forest trees were removed from the 1850's onwards. Between 1950 and the early 1980's an Exclusive Forest Permit (EFP) existed at the site and saw-log tree were removed. Since this time the site has been set aside as a research area and apart from the occasional illegal collection of firewood it has remained undisturbed.

The study site is bisected by a forestry road. From the road on the southern side the site slopes up hill and is characterised by open *Eucalyptus pulchella* forest with the occasional *E. viminalis* and *E. globulus.* The shrub layer is predominantly *Callistemon pallidus* and *Acacia mearnsii,* with sporadic *Banksia marginata.* The ground cover consists of saggs (eg *Lomandra longifolia)* and grasses. On the northern side of the road the site is divided into two main areas. The first is a flat area that extends north to a boundary fence and slopes down hill to the east and west. This area is *E. obliqua-. E. amygdalina* forest with a sparse understorey of *Acacia mearnsii* and occasional *Exocarpus cupressiformis* and *Eucalyptus* saplings. The ground cover comprises mainly grasses and saggs. The second area is a denser region of vegetation surrounding a creek that runs from north to south along the western edge of the study site. This area is *E. amygdalina-E.viminalis* forest with an occasional *E. brookerana.* The understorey consists of *Acacia stricta, A. mearnsii* and *Letospermum scoparium.* The ground cover comprises mainly *Lomandra longifolia, Lepidosperma grandis* and grasses. Along the creek-line itself the understorey is a dense impenetrable tangle of *Gahnia grandis, Leptospermum lanigerum* and *Acacia verticillata.*

4.2.2. Field Methods

4.2.2.1. Trapping

Mascot live wire traps (30x30x60cm) were used to capture the possums. The traps were not arranged in a grid or a random pattern, but were placed in areas that appeared to be used by possums, such as below trees with possum scratches up the trunk and beside pathways made by animals. This method was chosen to maximise the chance of capturing possums, rather than collect trapping data for analysis.

Traps were baited with apple before dusk and checked early the following morning. When not in use the traps were wired open to enable animals to enter and leave without being caught. This enabled the animals to become familiar with the traps with the aim of increasing trapping success. Between November and December 1992 40 traps (numbers 1-40) were used. In January 1993 another 10 traps were added (numbers 41-50) (see Figure 9).

Forty-six field trips were conducted between November 1992 and February 1995. Between November 1992 and November 1993 an average of 10.8 ± 4.1 days from each month, consisting of two field trips per month, was spent trapping animals and collecting data on individual possum movements and scent marking. From December 1993 through to February 1995, 5.1 ± 2.9 days per month were spent in the field. One trapping trip was conducted each month to continue collection of data on individual possums, with an additional trip in some months to measure habitat parameters (see § *4.2.2.3 Measuring home range and den sites* below).

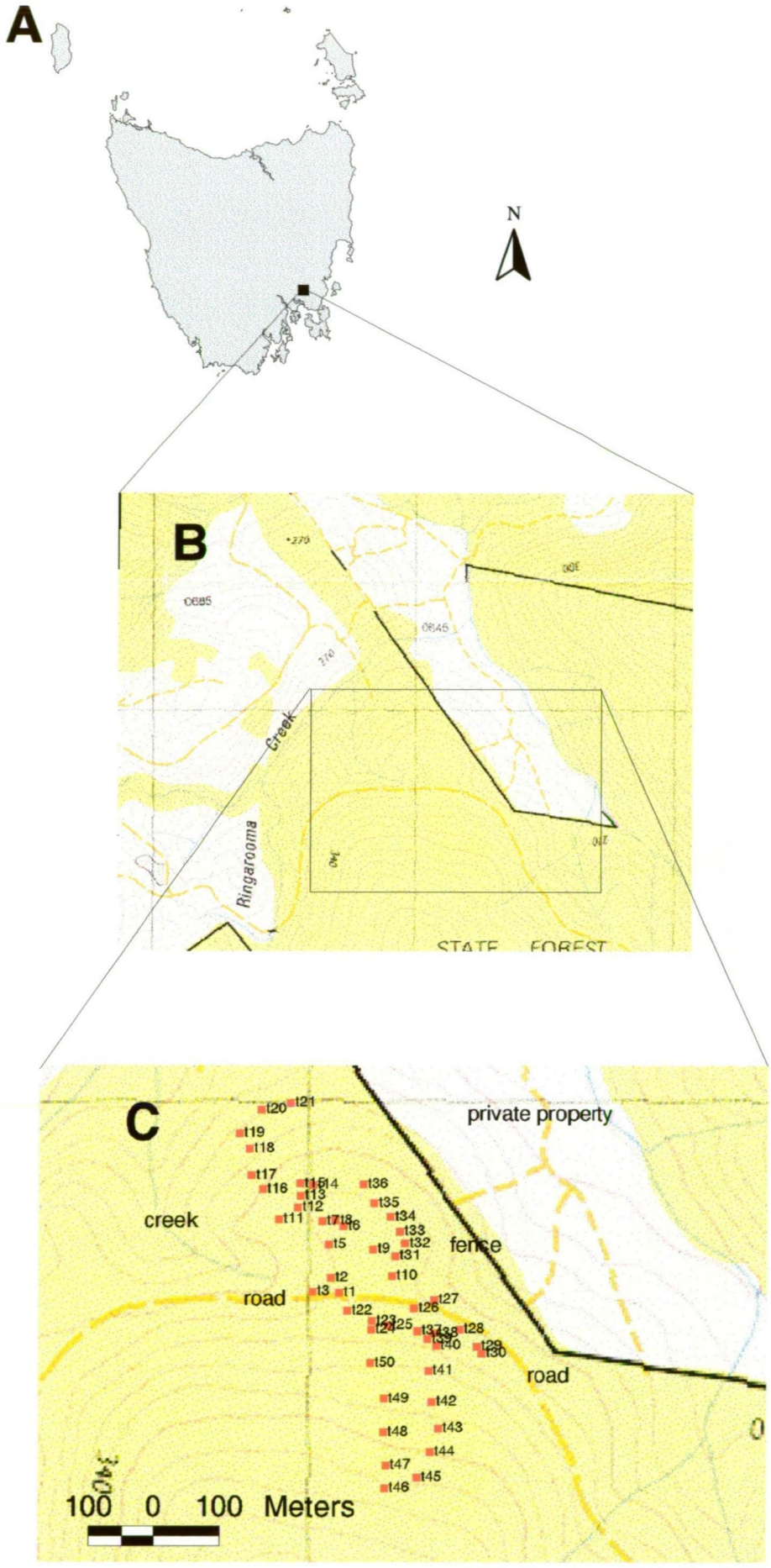

Figure 9.
A. Map of Tasmania indicating study area.
B. Section of the Mt Morrison State Forest.
C. The study site showing location of traps, road, creek and fence.

Plate 6. The study site: Mt Morrison State Forest, looking north.
(Photo: K. Hynes)

Plate 7. The study site: Mt Morrison State Forest, looking south.
(Photo: K. Hynes)

4.2.2.2. Handling and measurement

Captured animals were encouraged, by gently blowing on their bodies, into a pre-weighed hessian bag and then weighed (Salter 10kg balance). To sedate the animals for handling they were injected intramuscularly with a mixture of "Ketamine Injection" (ketamine hydrochloride, 100mg/ml, Parnell Laboratories (Aust) PTY. LTD.) and "Rompun" (xylazine hydrochloride, 20mg/ml, Bayer Australia LTD,) (Wright 1983). Animals were marked for individual identification by tattooing a two-digit number in the left ear.

A series of body measurements were made using Vernier callipers and a tape measure. For both sexes head, ear, manus, pes and tail length were measured using the methods of Lyne & Verhagen (1957). The length and width of fur stained by sternal gland secretion was also measured. An index of sternal staining was calculated by multiplying the length and width of the stained fur. A similar method, using the sum of the length and width of the raised integument, was used by Stoddart (1980c) to create an index of activity for the subauricular gland in bandicoot species.

For females, maturity and reproductive state was determined using the methods of Hocking (1981). Females were examined for the presence of a pouch, the condition of the pouch and nipples the presence of pouch young. Females without pouches and inverted nipples were considered to be immature. Females with pouches were classified as mature and were further divided into 4 groups:

- Mature animals in oestrus condition (ie pouch clean and moist with a waxy secretion and a slight reddening around the nipples).
- Mature animals carrying pouch young.
- Mature animals without pouch young but lactating.
- Mature animals in anoestrus (ie pouch noticeably drier).

For males the scrotal width and length and width of the left and right testis were measured. A number of ways of distinguishing between juvenile and adult males appear in the published literature. Gilmore (1969) and Smith *et al* (1969) observed that spermatozoa were absent from the testes of most possums with a dissected testis weight <2g. It was not possible to measure testis weight in males captured in this study. Tyndale-Biscoe (1955) showed a correlation between the presence of spermatozoa and the length of the testes. Spermatozoa were not found in males with a testis length $\leq$ 17mm. In a study of the histology of the sternal integument (see *Chapter 2. Histology of the Sternal Integument*) juvenile and adult male possums were distinguished by weight. Male possums less than or equal to 2 kg body weight were considered to be juveniles; individuals with a body weight greater than 2 kg was considered to be an adult. Reasons for choosing this cut off point are outlined in Chapter 2. Using weight to classify males in this field study revealed that all males with body weights greater than 2kg had testes lengths greater than 17mm. Testis length was not measured in the two males that weighed less than 2kg. Both these males were observed to have very small testes that were not completely descended. A comparison of males revealed that the average scrotal width of males weighing more than 2kg was significantly greater (t-test: $p << 0.001$), being twice that of males weighing 2kg or less. Based on these findings body weight was used to distinguish between sexually immature and mature males in this field study: males with body weights less than or equal to 2 kg were classified as immature and males weighing more than 2kg classified as mature.

Each of the measurements described above was made the first time an animal was captured during a field trip. Females caught more than once during a trip were examined for the condition of their pouches and the presence of pouch young each time they were captured.

4.2.2.3. Measuring home range and den sites

To determine the home range of individual possums, animals were fitted with either 3 or 4 reel spool-and-line tracking devices (see *Chapter 3. Recording Scent Marking in the Field*). Starting from the trap that the animal was caught in, a record of the possum's path was made by taking compass bearings each time the spool changed direction and pacing the distance of each directional segment. An effort was made to track each possum within the study site a least once each month — this was not always possible, however, as not all individuals were caught each month and occasionally a spool did not work successfully.

Location of nest sites were made in two ways — during spool-and -line tracking and by surveillance of radio collared animals. When following spools, trees that had spool-line going up them were examined for den entrances. Trees in which spool-line was clearly seen entering (and in some cases exiting) a hole in the tree were considered to be den sites. Evidence of other den sites (eg hollow logs) could also be determined from following the spool-lines. Between September and December 1994 four possums (2 males and 2 females) were fitted with collar-style radio-transmitters. During the day the den site of the animal was located using a handheld Yagi antenna and receiver. Where the den site was a tree a record of the species (if alive), diameter at breast height (DBH), tree height and height of den entrance was made.

To enable maps of the home range and location of nest-sites within the home range to be constructed, a geographical positioning system (GPS) technology (*Pathfinder Single Frequency GPS*, Trimble) was used to record the location of traps and trees within the study site.

4.2.3. Analysis of home range and den site use.

To map home ranges and den sites geographical information system (GIS) technology was used to display the GPS and spool-and-line data collected in the field. The software packages, *MapInfo* (MapInfo Corporation), *ArcView* (Environmental Systems Research Institute (ESRI), Inc.) and *ARC/INFO* (ESRI, Inc) were used.

The GPS location of traps and trees were entered into *MapInfo*. All spool-and-line data was added to the map using a purpose written "bearing and distance" programme (Landfile Consultancy Pty. Ltd.). This enabled the path of the possum to be mapped from a known GPS point (ie a trap). All further data manipulation was performed in *ArcView* and *ARC/INFO*. Home ranges were estimated by constructing a boundary around the area used by an individual possum. The boundary encompassed all the spool-and-line information, as well as the traps the possum was caught in and the trees they visited. Construction of a boundary enabled the area and perimeter of the home range to be calculated.

Before discussing the results it is important to note a number of limiting factors in the presentation of the home ranges of possums at the site.

Firstly, the area north of the fence was private property and not part of the study area. This area was a sparsely vegetated northeast-facing hill that sloped down from the fence for approximately 250m to a private road that runs parallel to the fence. On the opposite side of the road was a young (<1 year old at the start of the study) *Eucalyptus* sp. plantation on the flat valley floor. The road beside the plantation was periodically laid with 1080 poison over the duration of the study. Some spool-lines of a number of individuals extended over the boundary fence: Female F01 2/8 spools went over fence; female F02 12/14; female F03 6/12; male M04 1/3; male M09 5/15; male M12 2/3. Male M07 also crossed the fence once (spools: n=19) and was radio-tracked to a number of den trees over the fence between September and December 1994. (It should be noted that M07 had not been in this area at

all prior to Aug 1994; he started to extend his home range into this area following the disappearance of M09 in April 1994).

Because no access was granted to the private property it was not possible to record the path taken by spool-lines that extended into this area (except for a few that continued over the fence for a relatively short distance). Collection of spool-line over the fence revealed that all of the animals venturing into this area moved between 100 and 250m down the hill towards the road. It was possible, however, to collect information on the den sites used by individuals in this area (from the spools and radio-transmitters). Despite the lack to detailed information for spool-lines that extended over the fence it was still possible to construct home ranges for these animals using the den site data. It should be noted, however, that the sizes of the home ranges of animals that used this area on a regular basis might be underestimated.

Secondly, three possums (F07 and F08 and M08) had home ranges that extended past the western boundary formed by the creek. Due to the impenetrable nature of the vegetation surrounding the creek it was impossible to track animals once they moved into this area. Female F07 was tracked five times, three of which she went into the creek vegetation. Possums F08 and M08 were each tracked once into the creek. The decision was made not to track these animals again as their movements into the creek vegetation limited the information that could be gained.

Thirdly, the eastern side of the study site was adjacent to a firewood-cutting lease. Due to the high level of disturbance in this area as a result of selective tree cutting no data were collected on animals once they ventured into this area. Male M04 was the only animal recorded in this area.

In general the results in this chapter will be limited to those animal with adequate home range data, that is, animals that were caught on a regular basis (at least once a month) and were tracked using spool-and-line at least once every two months (NB most were tracked at least once per month) over a period of a least six months duration. Individuals meeting these criteria are listed in Table 29. Two additional females (F06 and F07) are shown at the bottom of the list; although they do not meet all the criteria they have been included as they will be discussed with respect to some of the findings.

Another important observation that warrants mentioning at this point is that not all individuals were caught throughout the study period. A number of possums caught regularly (ie listed in Table 29) disappeared from the study site. Male M01 was present between November 1992 and May 1993 only. Male M09 was not trapped until May 1993; he was then caught regularly until April 1994, after which he disappeared. Among the females, F01 was captured between January and August of 1993 only. Changes in the home ranges of adjacent possums were observed following the loss an individual. The impact of the loss of each of the individuals listed above is discussed.

4.3. Results

4.3.1. Home range

4.3.1.1. Home ranges of male and female possums.

Details of the trapping, spool-and-line tracking and home range size of individuals caught on a regular basis are shown in Table 29. Male home ranges are significantly larger (t-test: $p = 0.009$) than female home ranges (mean ± sd: males (n=3) = 5.2 ± 1.1 ha; females (n=5) = 2.3 ± 1.0ha).

Note: Four home range areas are shown for male M07. The first is the total area used by M07 during the study. This home range consists of three ranges (shown under the first) which correspond with changes in the home range of M07 following the disappearance of M01 and then M09 during the study. Further explanation is given in *§4.3.1.3 Changes in individual home ranges.*

Table 29. Trapping, spool-and-line tracking and home range data for individual possums.

Animal	Trapping period		No. of times trapped	No. of spools	Area (ha)	Perimeter (m)
M01	Nov '92 – May '93	(7 months)	17	11	4.2	1676
M07	Feb '93 – Feb '95	(25 months)	27	19*	6.4	2204
	(Feb '93 – May '93	M01 present)	4	2	0.9	797
	(Jun '93 – Apr '94	M01 gone, M09 present)	12	10	2.9	1560
	(May '94 – Feb '95	M09 gone)	11	7	0.4	2219
M09	May '93 – Apr '94	(12 months)	22	13	5.1	2273
F01	Jan '93 – Aug '93	(8 months)	16	8	1.7	1864
F02	Jan '93 – Oct '94	(22 months)	29	14	3.2	1180
F03	Jan '93 – Jan '95	(25 months)	37	12‡	3.6	1306
F06	May '93 – Sep '93	(5 months)	10	3	1.4	848
F07	Jun '93 - Sep '94	(16 months)	11	5	1.6	936

*M07 last spool-line in November 1994

‡F03 immature between January 1993 and early November 1993 (3 spool-lines), from late November 1993 to March 1994 approaching sexual maturity (pouch forming, weight increased, started scent marking) (2 spool-lines), and mature from April 1994 onwards (7 spools); last spool-line in October 1994.

The home ranges of all males (n=17) and all females (n=9, NB 3 juveniles not shown) caught during the field study are shown in Figures 10 and 11 respectively. Animals caught once are shown as squares rather than boundary outlines.

Among males there appears to be a large degree of overlap in the range of individuals. Much of this overlap is due, however, to individuals moving into the range of another animal that had disappeared from the study site. This is examined in greater detail in *§4.3.1.3 Changes in individual home ranges.* Taking the changes in home range following the disappearance of individuals into account there is very little overlap of male ranges.

There is very little overlap between the home ranges of female possums. The extensive overlap between females F01 and F03 (show in Figure 11) is examined in *§4.3.1.3 Changes in individual home ranges.*

Although there is little overlap in home range within each sex there is a great deal of overlap between the sexes. Figure 12 shows the overlapping pattern of all male and female home ranges. Figures 13 and 14 show the home range overlap of adjacent males and females before (Figure 13) and after (Figure 14) the disappearances of M01 and F01.

In Figure 13 M01 can be seen overlapping with females F01 and F02. Most of the home range of F01 is found within male M01's home range; only a small amount of females F02's range occurs within male M01's range. The home range of male M07 also overlaps with the home range of female F02. Following the disappearance of female F01 and male M01the pattern of male-female range overlapped changed. In Figure 14 it can be seen that M07's range expanded to include more of female F02's range and now includes some of the range of female F03, who has expanded her home range into that previously occupied by F01. Male M09 had moved into the area and has a range that overlaps with females F03 and F06. Both males and females have ranges that overlap with more than one individual of the opposite gender.

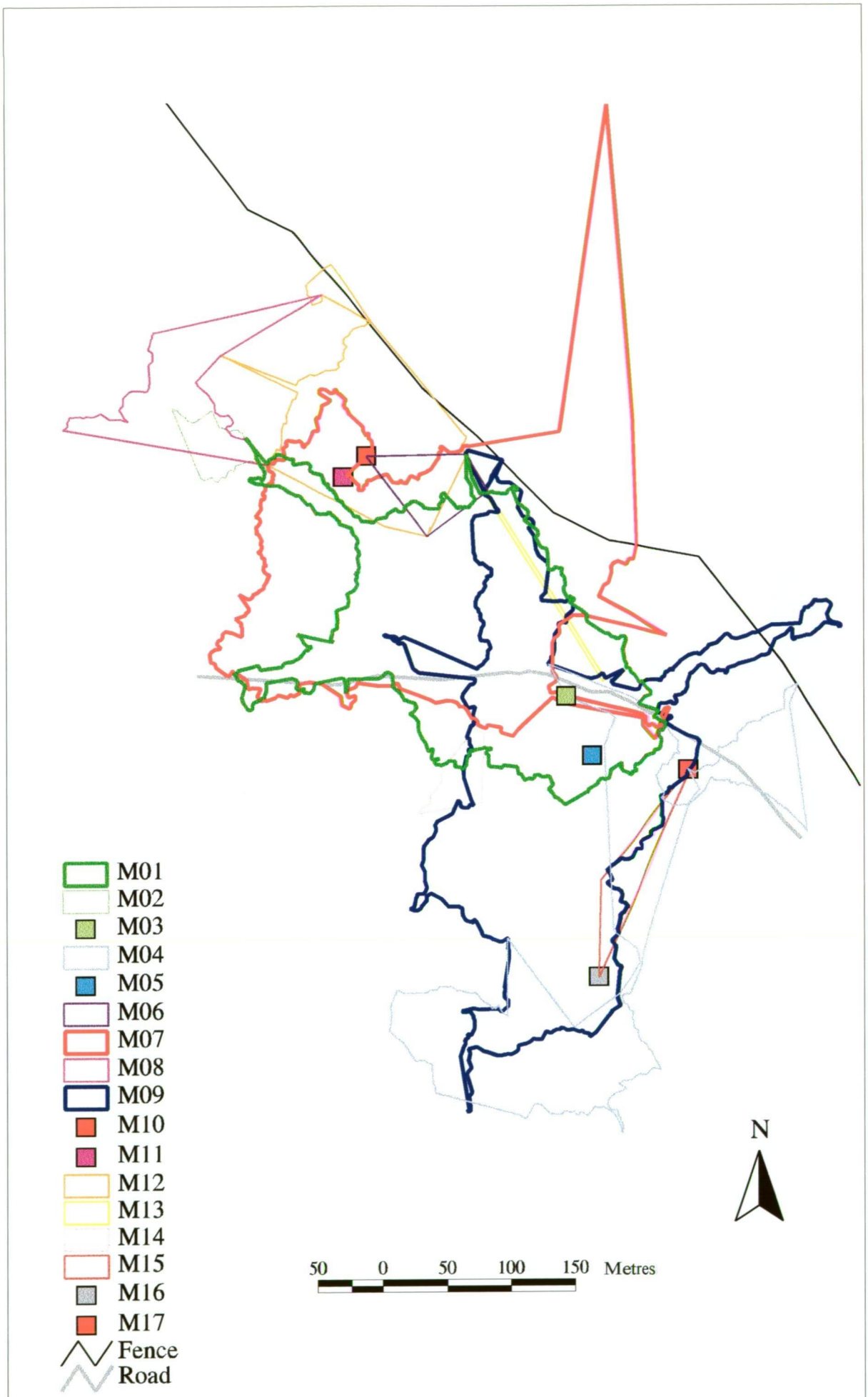

Figure 10. Home ranges of all male possums (Nov '92- Feb '95)

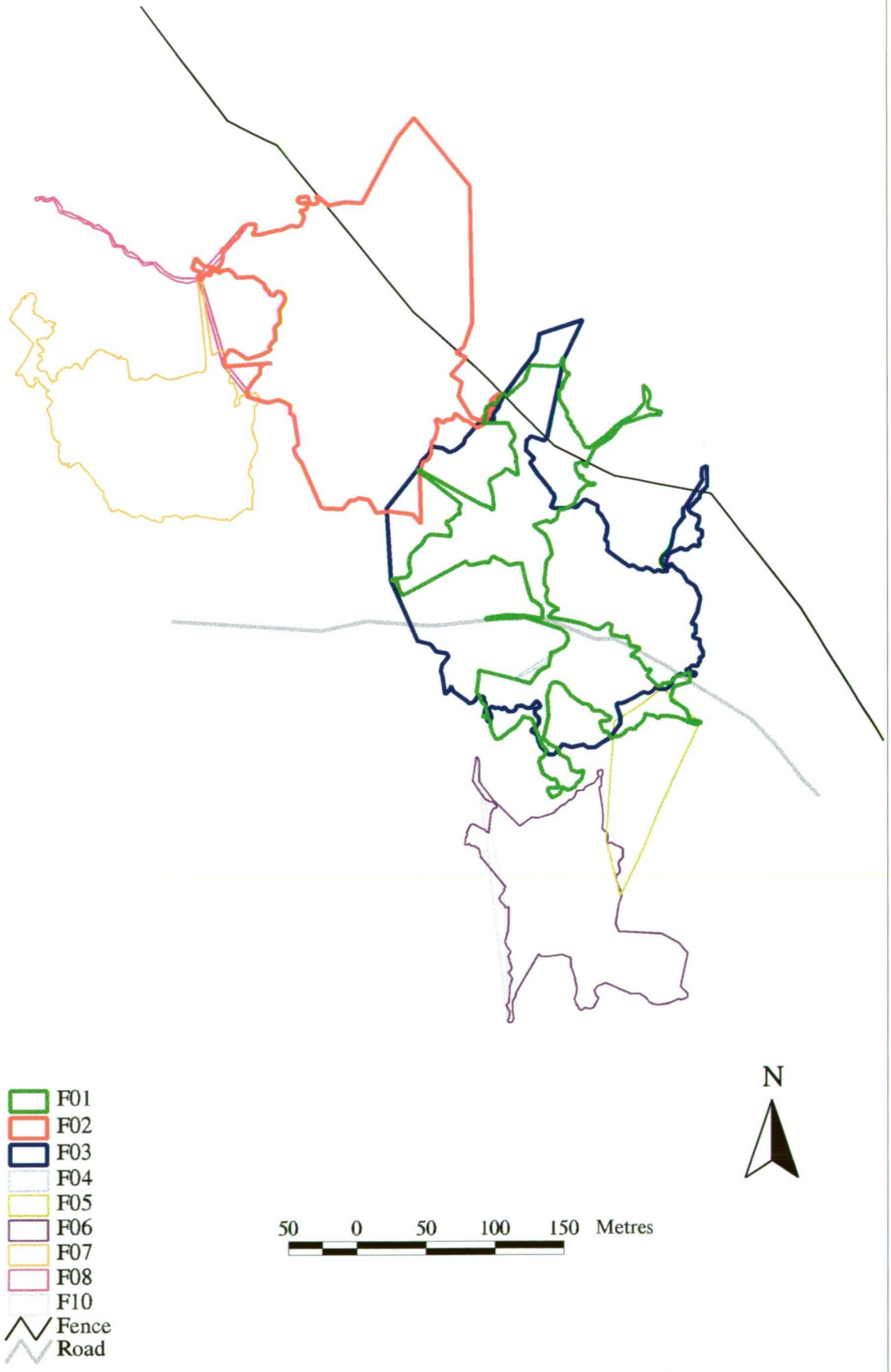

Figure 11. Home ranges of all female possums (Jan '92- Jan '95)

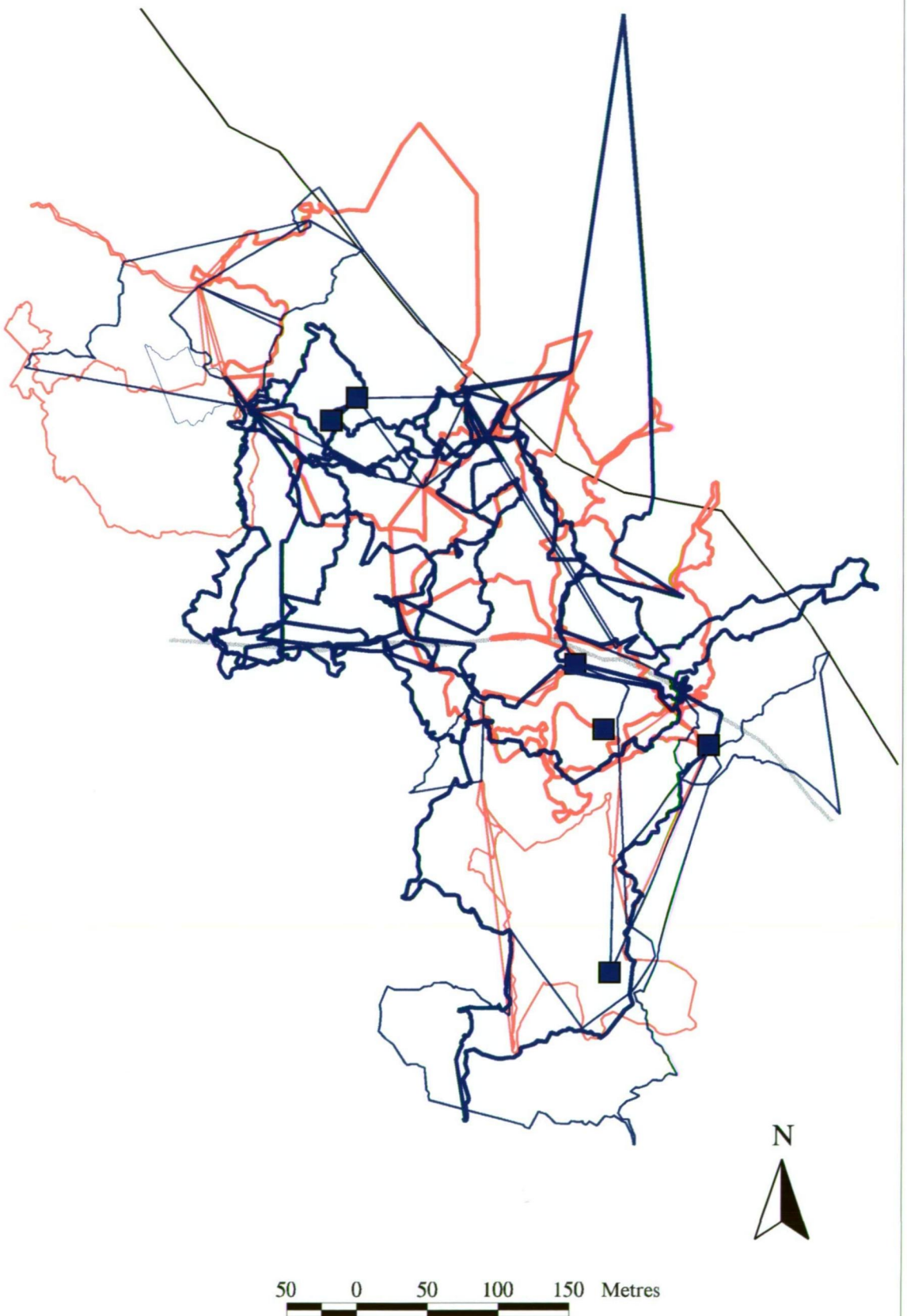

Figure 12. Home ranges of all male (n=17, BLUE) and female (n=9, RED) possums (Nov '92- Feb '95)

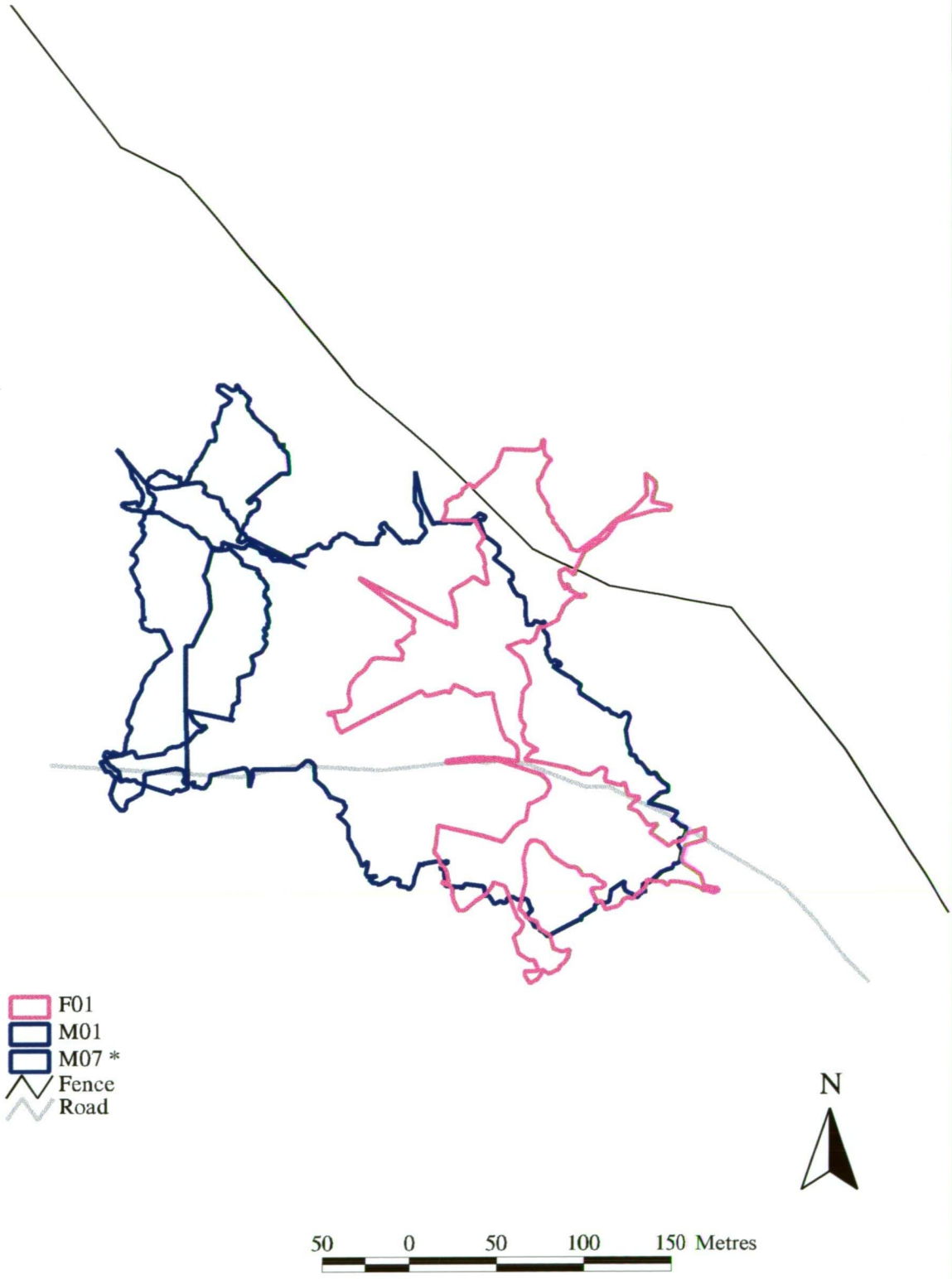

Figure 13. Home ranges of adjacent male and female possums

* The home range shown for M07 is the area used by M07 when M01 was present.

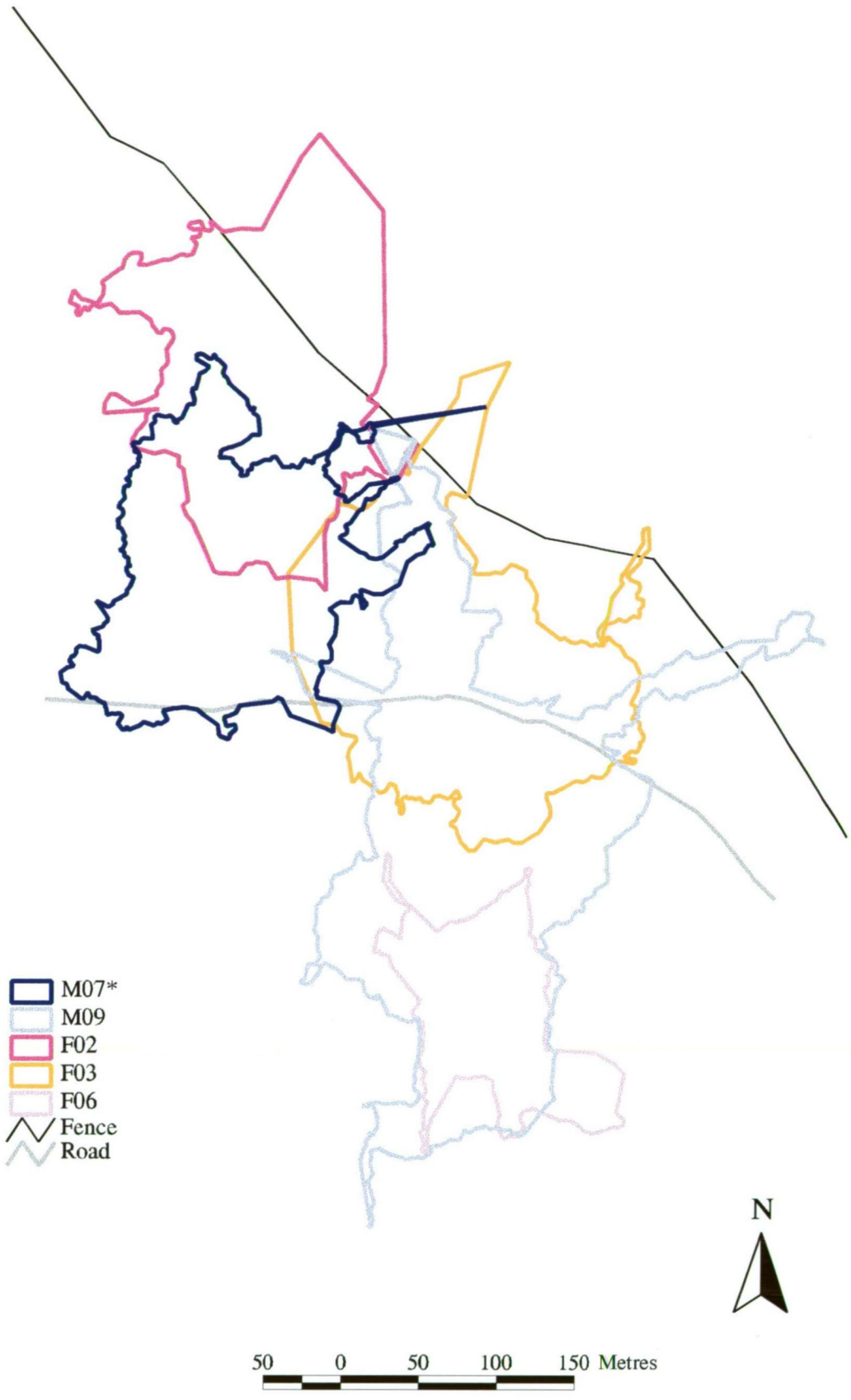

Figure 14. Home ranges of adjacent male and female possums (after the disapperance of M01 and F01)

* The home range shown for M07 is the area used by M07 after M01 disappeared, but M09 was present.

Examination of the individual possums captured during the field study reveals that within each gender a number of distinct groups exist.

The 17 males caught can be divided into three major groups. The first group (Group A in Table 30) consist of six males that were captured on a regular basis (usually at least once a month) during the field study. All six males were mature adults (see Chapter 2 for definition of maturity in adult males). Three of these males, M01, M07 and M09, were examined using spool-and-line tracking. The remaining three, M04, M08 and M12 had home ranges that extended outside the study site. The second group of males (Group B in Table 30) consists of five individuals that were caught once only during the study period. All five males were mature. Four of these males were caught between the months of November and January. The remaining male was caught in August. The third group of males (Group C in Table 30) consists of a mixture of juveniles and adults caught between 2 and 6 times over varying periods of time. Males M02 and M13 were both caught twice over a two-month period. M02 was caught between November and December and was a juvenile animal; M13 was caught between September and October and was an adult. The remaining four males, M06, M10, M14 and M15, were captured irregularly over periods ranging from 7 to 15 months. M06 was a juvenile during the 7-month period he was captured. The other three males were all adults.

There were no significant differences in body weight, scrotal width, testes length or sternal staining index among the mature males from each group, except for testes length between males in Group A and Group B. Males in Group A had significantly larger testes lengths than Group B males (t-test: $p = 0.009$). Juvenile males in Group C had significantly lower body weight, scrotal widths and sternal staining than all three mature male groups.

The 11 females caught during the study can be divided into two main groups: adult and juvenile individuals. (It should be noted that females F03 and F08 are included in both the mature and immature female groups because these individuals achieved sexual maturity during the study). Within the mature females two groups can be identified. Group A in Table 31 consists of mature females that were caught regularly (ie at least once per month, F01, F02, F03) during the study period. Groups B consists of mature females that were caught for only a short period of time (ie 6 months or less; F06, F08 and F10) or were trapped for more than 6 months, but less frequently than once a month (ie F05 and F07). There was no significant difference in the body weight of the two mature groups of females (t-test: $p = 0.290$), but the sternal staining index of the females in Group A was significantly larger than in Group B (t-test: $p << 0.001$).

Among the Group A and B females there were five individuals (ie F01, F02, F03, F06 & F07) for which there were good data on their reproductive success. Seven young were born to these females during two autumn breeding seasons. There were no observations of spring breeding in either year, even though there were females that had lost their autumn born young. Of the seven young born during the study only two survived past pouch life and were observed riding on their mother's back. The remaining five young survived for between two and four months only. There was an obvious difference between the successful and the unsuccessful mothers. Firstly, the females who reared their young past the pouch life stage maintained and actually increased their body weights (250-300g) over the winter months when the young was in the pouch. The unsuccessful females all lost considerable amounts of weight, between 350 and 1250g, which was between 12 and 43% of their pre-pouch young weight. The successful females were F01 in 1993 and F03 in 1994, both were Group A females. It is interesting to note that the successful females occupied the same habitat; female F03 resided within F01's home range as a juvenile and expanding into the whole range following the disappearance of F01 August 1993.

Among the immature females there were two groups. Females F09 and F11 (Group C) were each caught once outside the pouch only during the study — both were trapped with their mothers (F05 and F03, respectively) and were observed to be riding on her back. F09 and F11 were approximately 8 and 6 months of age respectively when caught. Neither of

these females was caught independent of their mother later in the study. The remaining three immature females (Group D: F03, F04 and F08) were caught without their mothers. These juveniles were older, having significantly higher body weights (t-test: $p << 0.001$) and larger sternal staining indexes (t-test: $p = 0.016$) than the dependent juveniles. Two of these juveniles (F03 and F08) remained in the study site and reach sexual maturity. The other juvenile, F04, was only captured twice over two days in January.

Table 30. Characteristics of male possums.

The mean ± SD for body weight, scrotal width, testes length and sternal staining index is given for each group of males.

	No. of times trapped	Duration of trapping	Body weight (g)	Scrotal Width (cm)	Testes Length (cm)	Sternal Staining Index (cm^2)
Group A			2849.4 ± 367.4	4.3 ±0.4	2.3 ± 0.4	42.2 ± 10.0
M01	17	Nov '92 –May '93 (7 months)				
M07	27	Feb '93 – Feb '95 (25 months)				
M09	22	May '93 – Apr '94 (12 months)				
M04	9	(Jan '93 1x) Sep '93 – Dec '94 (24 months)				
M08	15	May '93 – Aug '94 (16 months)				
M12	17	Aug '93 – Oct '94 (15 months)				
Group B			3250 ± 663.3	4.2 ± 0.7	2.1 ± 0.2	45.3 ± 5.8
M03	1	Jan '93	* 2987.5 ± 356.8			
M05	1	Jan '93				
M11	1	Aug '93				
M16	1	Nov '94				
M17	1	Dec '94				
Group C Juveniles			1550 ± 300.0	2.0 ± 0.3	no data available	20.5 ± 8.1
M02	2	Nov – Dec '92 (2 month)				
M06	6	Feb – Aug '93 (7 months)				
Group C Adults			2675 ± 391.8	4.2 ± 0.6	2.1 ± 0.2	41.8 ± 11.6s
M10	3	Jul '93 – Sep '94 (15 months)				
M13	2	Sep '93 – Oct '93 (2 months)				
M14	2	Nov '93 – Sep '94 (11 months)				
M15	3	May '94 – Nov '94 (7 months)				

* Not including the body weight of M17 which, at 4300g, was 700g more than any other male caught during the whole study.

Table 31. Characteristics of female possums.

The mean ± SD for body weight, and sternal staining index is given for each group of females.

	No. of times trapped	Duration of trapping		Body weight (g)	Sternal Staining Index (cm^2)
All Adults				2548.1 ± 236.3	36.6 ± 9.1
Adults:					
Group A				2572.2 ± 212.0	39.5 ± 10.0
F01	16	Jan '93 – Aug '93	(8 months)		
F02	29	Jan '93 – Oct '94	(22 months)		
F03	18	late Nov '93 – Jan '95	(15 months)		
Group B				2514.1 ± 266.5	31.1 ± 7.9
F05	10	Apr '93 – Jan '95	(22 months)		
F06	10	May – Sep '93	(5 months)		
F07	11	Jun '93 - Sep '94	(16 months)		
F08	4	May – Nov '94	(6 months)		
F10	3	May – Jun '94	(2 months)		
Juveniles:					
Group C				725.0 ± 388.9	2.5 ± 3.5
F09	1	Dec '93			
F11	1	Nov '94			
Group D				1728.1 ± 195.8	21.8 ± 9.8
F03	19	Jan – early Nov '93	(11 months)		
F04	2	Jan '93	(2 days)		
F08	5	Jun – Dec '93	(7 months)		

4.3.1.2. Home range use

Spool-and-line tracking clearly shows the way individuals utilise the whole of their home range. Figure 15 shows the use of home range by males M07 and M09 and Figure 16 shows the same for females F02 and F03. By using spool-and-line tracking and mapping the paths taken by individuals over a period of time a number of things are evident. Firstly, most of the area in the home range is used; there are very few areas within the boundary of the home range that have not be covered by the resident animal. "Spooling" individuals for a longer period of time may have shown that areas not covered are also used. The maps show that in some areas spool lines overlap more than in other area. This overlap indicates that individuals often use the same path to move through their home range and that some areas of the home range are used more often than other areas. In Figure 15 two spools (one for M07, one for M09) are highlighted to show how large are the areas of the home range that can be covered in one night.

Use of spool-and-line provided information on the distances covered by individual possums during the night. The majority of the spools ran out before an animal returned to a den site, however. Only 2/56 male and 3/43 female spool-lines ended in a diurnal den site. Only one male, M07, was tracked to a diurnal den; he travelled 540m and 816m from the trap site to each den. Two females, F02 and F03 were tracked to diurnal den sites. Female F02 was tracked twice, although the distance she covered is only available for one site (ie 466m); the other den was located outside the study area and the length of spool to the den could not be calculated. Female F03 was tracked once to a diurnal den; she travelled 551m from the trap to the den. The low number of possums tracked to diurnal den sites indicates that most individuals were travelling more than 960m, the length of a 4-reel spool, during the night.

4.3.1.3. Changes in individual home range

Changes in the home range of animals following the disappearance of neighbouring animals were seen on a few occasions. Details of the effects observed are outlined below.

Male M01 was not caught after May 1993. When M01 was present M07's range bordered the western side of M01's range — there was only a small amount of overlap between the two individuals. After M01 disappeared from the study site two major changes were noted. Firstly, the next time M07 was tracked, and from then onwards, he expanded his range to include some of the western part of M01's range. The eastern part of M01's range was taken over by a new male, M09, who was not captured until after M01 had gone. The home range of males M07 and M09 before and after the disappearance of M01 are shown in Figure 17.

A similar result was observed when M09 disappeared in April 1994. Male M07 again increased the size of his range to include part of the northern section of M09's range. On this occasion, however, M07 appeared to be spending less time in the western most part of his range (ie the area he occupied when M01 was present). Changes in the home range of M07 with and without the presence of M09 are shown in Figure 18.

Among the female possums a similar situation was observed. Female F01 was present from the beginning of the study until August 1993. During this time her home range encompassed the range of an immature female, F03. It is possible that F01 was the mother of F03 — both Dunnet (1964) and Crawley (1973) recorded young females forming permanent home ranges close to the areas in which they were born. After F01 disappeared, F03 expanded her small home range into all of F01's range. Changes in the size of female F03's home range with and without F01 are shown in Figure 19. The range of female F02, situated to the north-west of female F01, did not appear to change following the F01's disappearance.

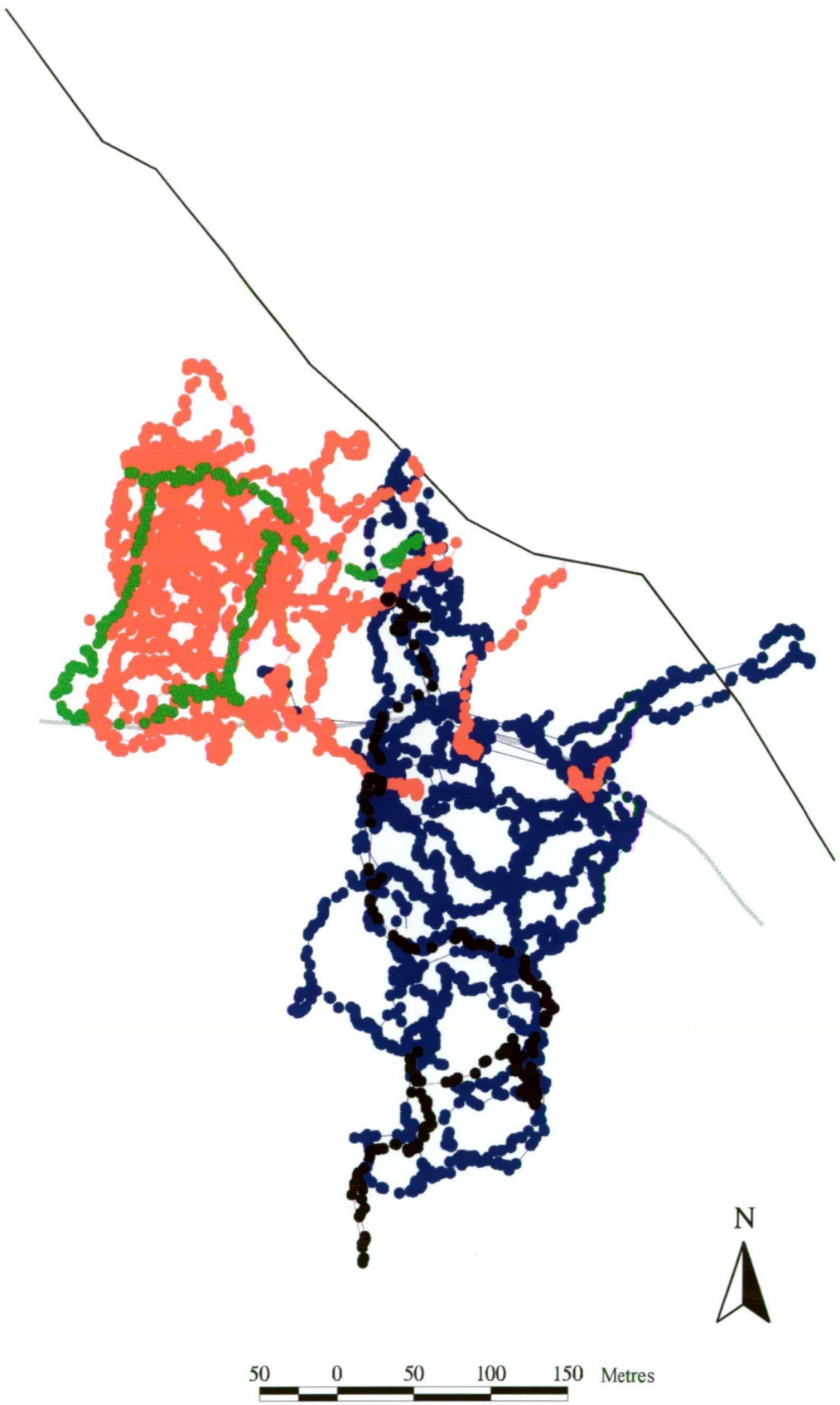

Figure 15. Home range use as indicate by spool-and-line tracking for M07 (n=19, RED) and M09 (n=14, BLUE)

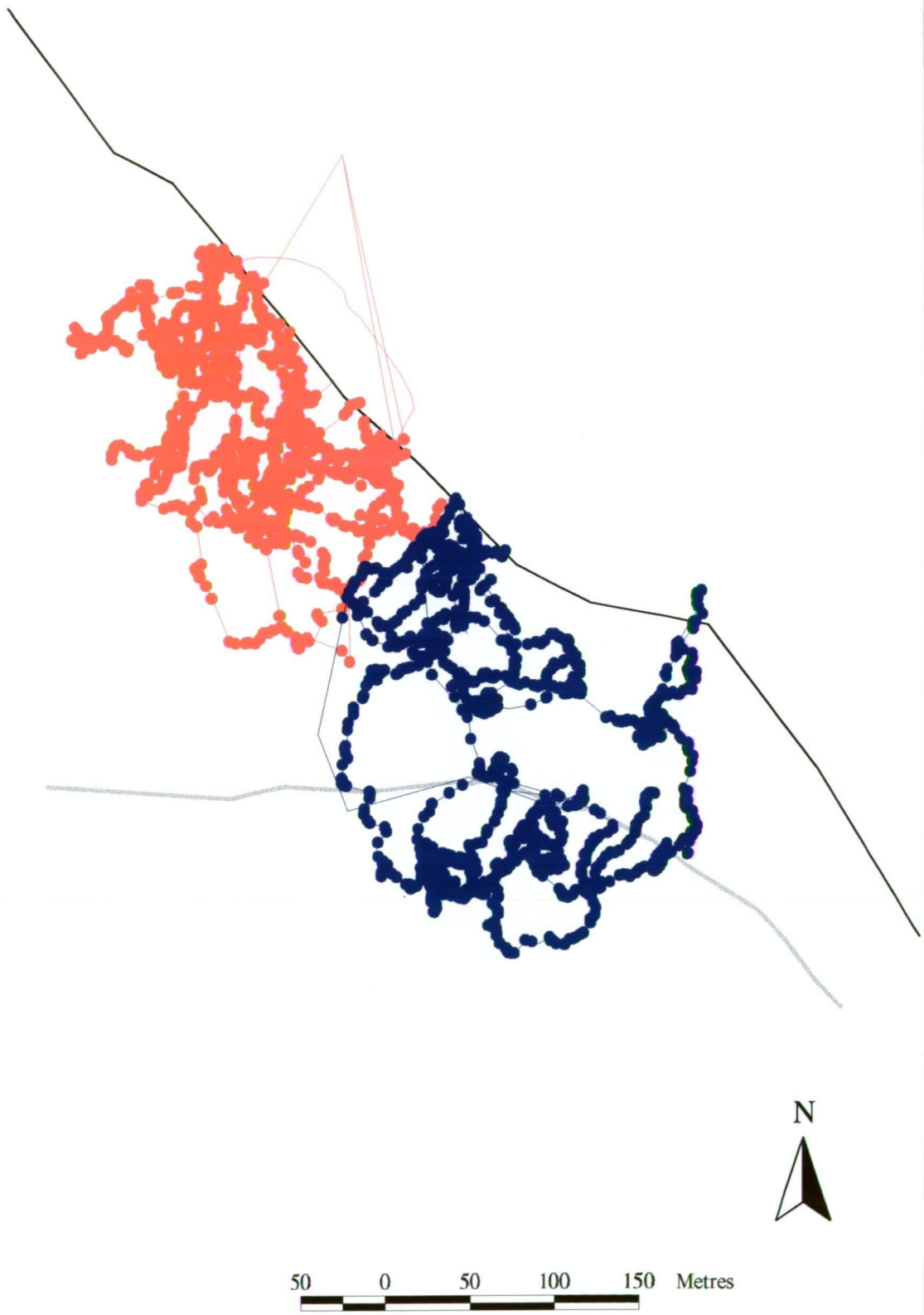

Figure 16. Home ranges use as indicated by spool-and-line tracking for F02 (n=14, RED) and F03 (n=12, BLUE)

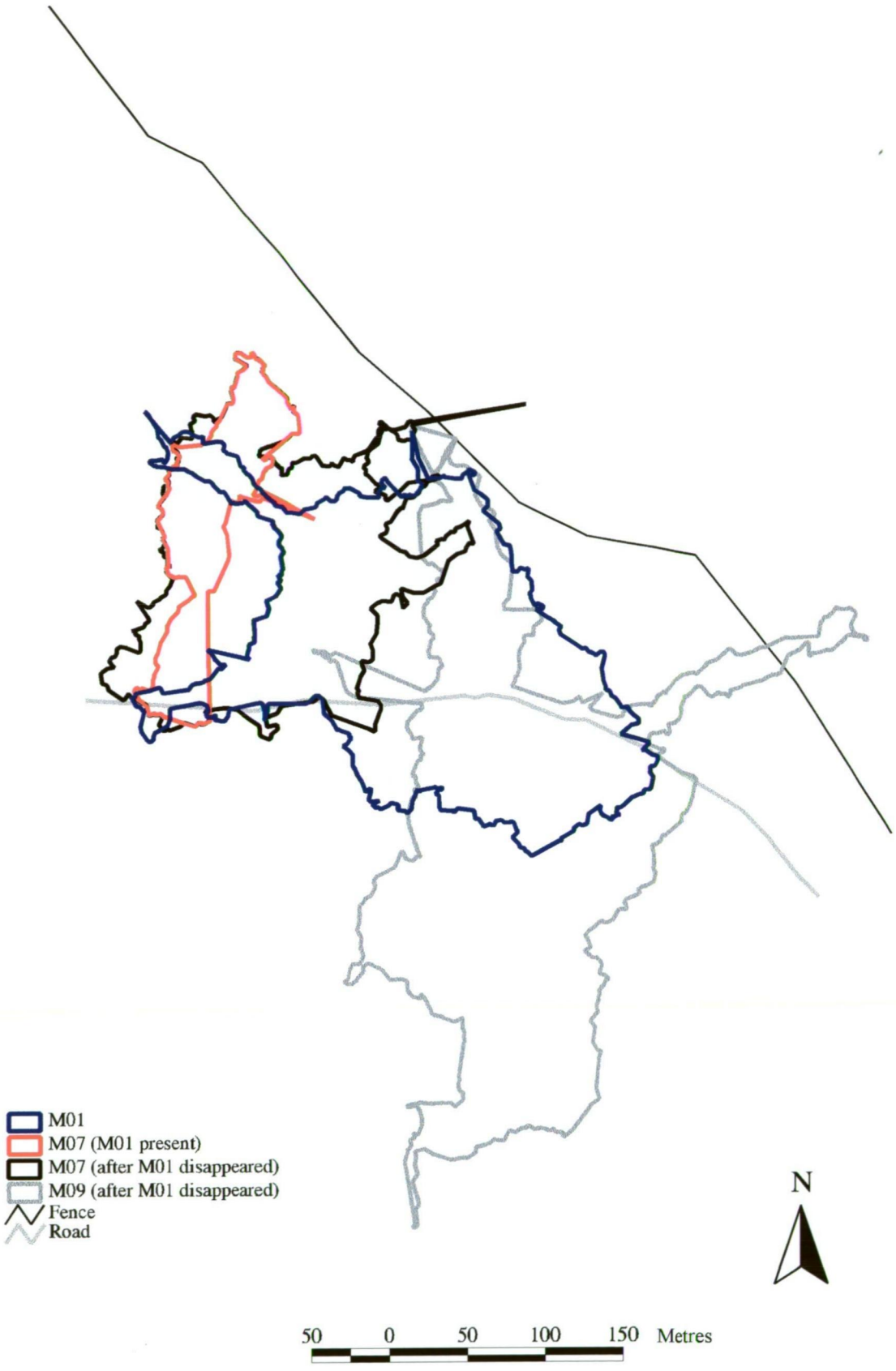

Figure 17. Home ranges of M07 & M09, with and without M01.

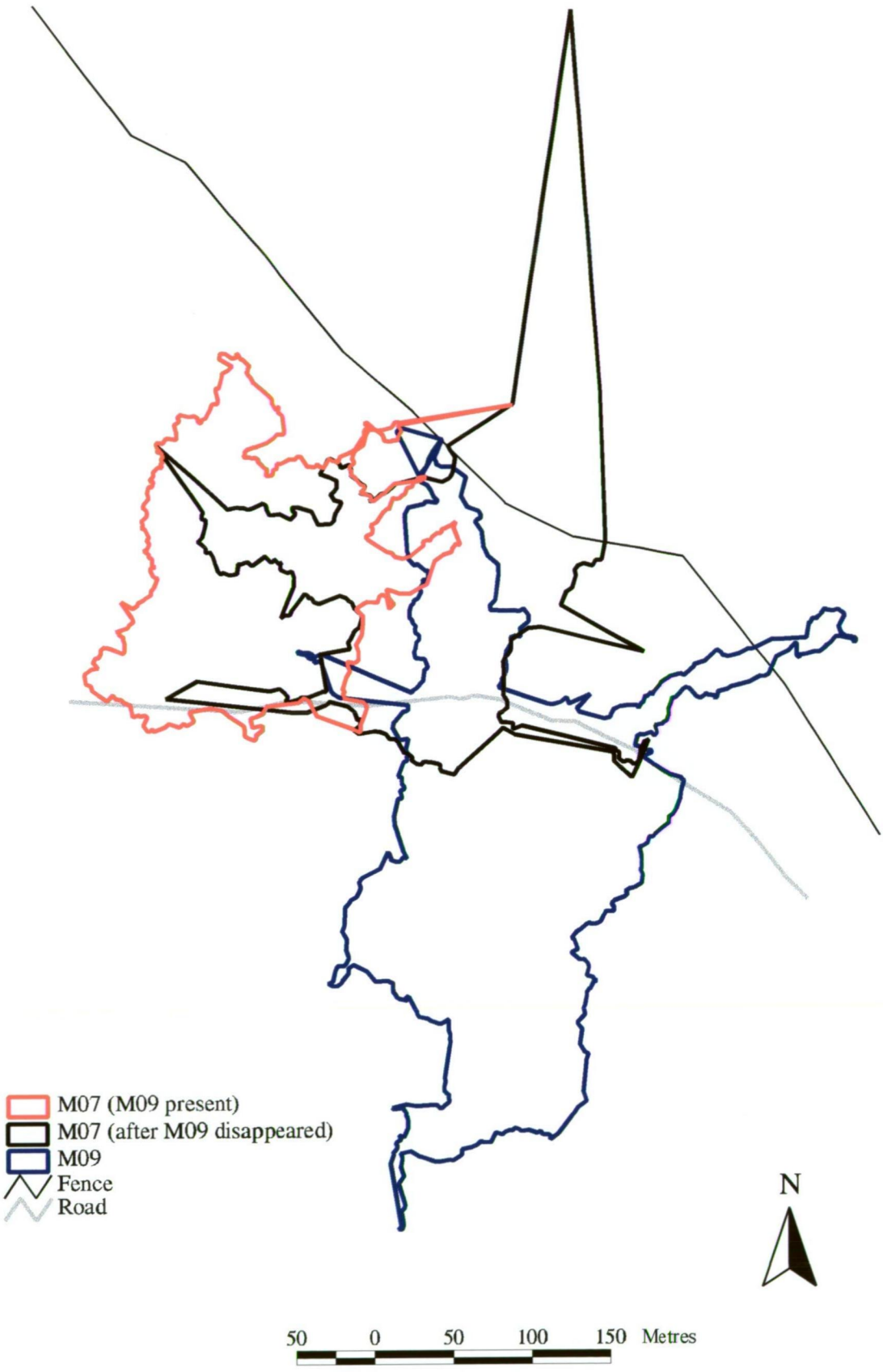

Figure 18. Home range of M07, with and without M09

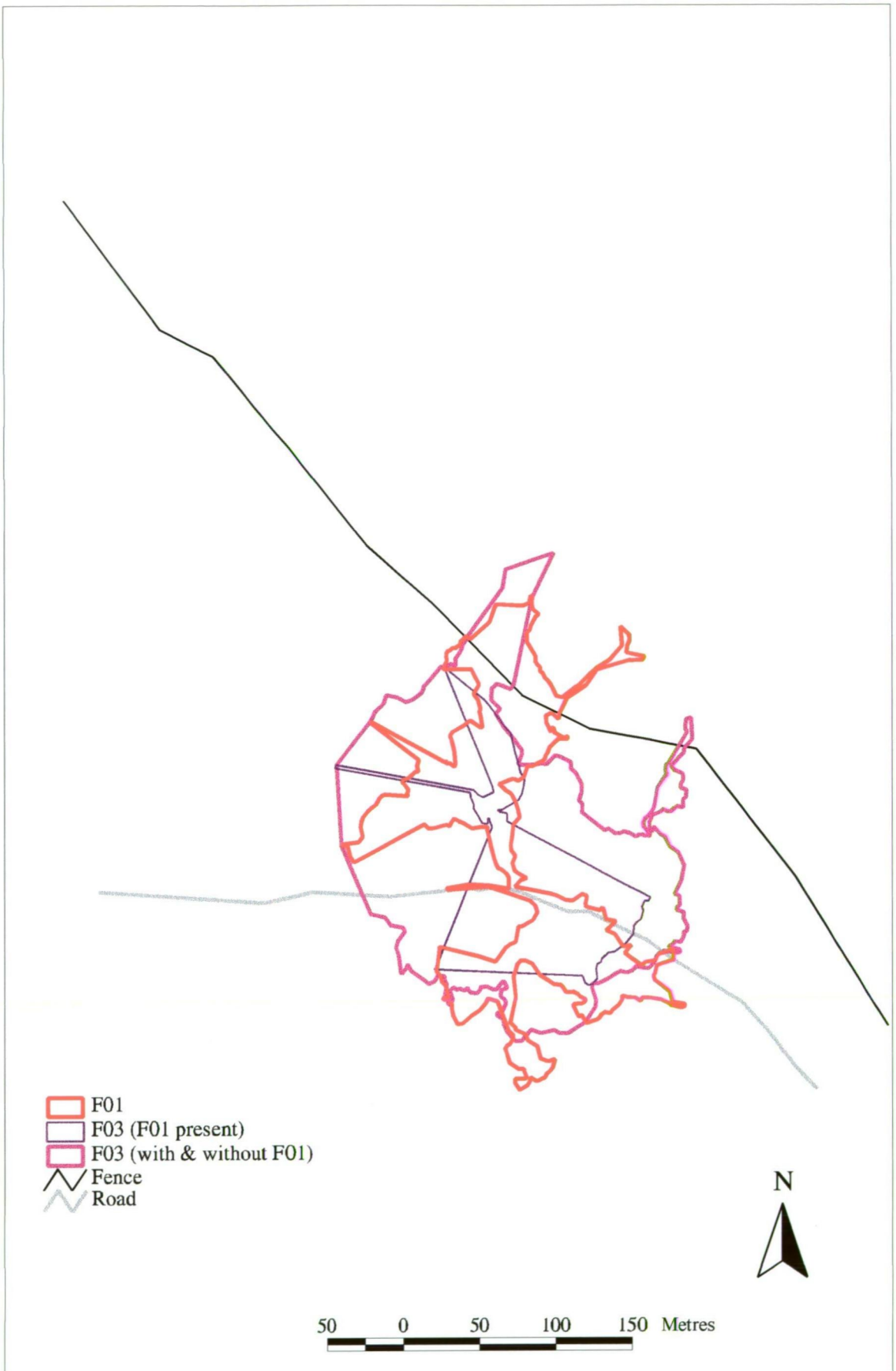

Figure 19. Home range of F03, with and without the presence of F01.

4.3.2. Den sites

Between November 1992 and February 1995 11 possums (6 ♂♂ & 5 ♀♀) were recorded in 35 den sites on 82 occasions. Eighty percent (n=28) of the den sites were trees and the remaining twenty percent (n= 7) were logs/fallen trees. Of the 28 trees, 23 (82.1%) were living and 5 (17.9%) were dead. Table 32 groups the tree den sites by species and gives details of their height, DBH and the height of the den entrance. Dens were found in all species of *Eucalyptus* except *E. globulus* and *E. brookerana,* which were uncommon at the site. Only trees with diameters of 50cm and above and heights greater than 14m were used as den trees.

The majority of den sites used by possums in this study were located off the ground. All the sites on the ground were in logs, although a number of trees had hollow bases which served as entrances to den sites higher up the trunks (determined from spool lines travelling up the hollow trunk). Both male and female possums were observed to use hollow log as a den site during the night at times when it was known to have rained. Only one male and one female possum were found using den sites located on the ground during the day. The female (F06) was observed over two consecutive days using a very exposed den site in a log. She was in very poor condition and probably died soon after these observations, as she was not trapped again in any subsequent field trips. Over a period of 3 months before using the log as a diurnal den she had lost 750g (28% of her body weight), 400g in the preceding 4 weeks. She had also lost her 3-5 week old pouch young, had a large weeping sore at the base of her tail and was visibly "bony". The male observed to use logs as a diurnal den sites was M07. In October 1994 male M07 used two different logs over a three-day period. At this time he was noted to have lost some weight (250g, ~8% of his body weight over the preceding 2 months), he had a number a large ticks and fleas, felt bony, and had fur missing from the dorsal surface between his tail and left hind leg. In the following month his weight dropped another 100g, but then increased by 600g in the next month. It is probable that the reason these individuals were using dens on the ground was because they were too weak to climb up into elevated den sites.

Use of individual dens by possums is shown in Tables 33 and 34. Maps of the location of den sites within the home range of individual male and female possums are shown in Figures 20 and 21 respectively.

Table 32. Characteristics of trees used as den sites: species, tree height, DBH and height of den entrance (range, mean ± SD).

Species	Tree Height (m) (L= height of living foliage; D= height including dieback)		DBH (cm)		Den Entrance Height (m)	
E. pulchella (n=12)	L (n=12): (12.1-31.7)	20.3 ± 6.1	(62.0 - 126.7)	85.8±20.6	(base - 15.7)	8.5± 3.2
	D (n=4): (12.7 - 33.7)	23.3 ± 8.7				
E. viminalis (n=6)	L (n=6): (8.0-31.9)	16.9 ± 9.4	(60.0 - 95.5)	73.2 ± 13.6	(base - 13.3)	10.6 ± 3.0
	D (n=3): (11.6 - 28.2)	17.5 ± 9.3				
E. amygdalina (n=3)	L (n=3): (22.1 - 26.4)	23.9 ± 2.2	(58.6 - 86.7)	72.0 ±14.1	(6.3 - 16.2)	9.8 ± 5.6
E. obliqua (n=2)	L (n=2): (23.7 - 28.9)	26.3 ± 3.7	(108.3 - 111.7)	110.0 ± 8.9	(base - 8.93)	4.5 ± 6.3
	D (n=1): (24.5)	24.5				
Dead (n=5) species unknown	D (n=5): (11.0 - 17.3)	14.4 ± 2.3	(49.8 - 75.3)	68.5 ± 13.0	(6.7 - 13.7)	9.6 ± 2.6
ALL TREES (n=28)	L (n=28): (8.0 - 31.9)	20.4 ± 6.9	(49.8 - 126.7)	80.3 ± 19.2	(base - 16.2)	9.4 ± 3.2
	D (n=13) (11.0 - 33.7)	18.6 ± 7.3				

Table 33. Den site use.

S = information gained from spool-and-line tracking; RT = information gained from radio tracking (Animals F02, F03 and M07 only).

Den ID	Date	Used by:	Possibly used by:*	Data
3	12/4/94	-	F03	S
	14/4/94	F03	-	S
	26/6/94	F03	-	S
	17/9/94	F03	-	RT
24	18/12/92	M01	-	S
	26/10/93	F03	-	S
	29/6/94	-	F03	S
29	17/12/92	M02	-	S
38	20/1/93	F02	-	S
58	25/2/93	-	F01	S
	8/6/94	-	M07	S
	15/9/94	M07	-	RT
	17/9/94	M07	-	RT
	12/10/94	M07	-	RT
	9/11/94	M07	-	RT
73	27/10/93	F03	-	S
	29/6/94	M07	-	S
	14/9/94	M07	-	S
99	20/4/93	M01	-	S
	23/7/93	-	M07	S
	16/9/93	F02	-	S
	13/10/93	F02	M07	S
	27/10/93	-	M07	S
	10/5/94	-	M07	S
	15/10/94	F02	-	RT
110	8/5/93	M01	-	S
	28/9/93	-	F07	S
	8/3/94	-	F07	S
176	19/6/93	M09	-	S
195	23/7/93	-	M07	S
237	15/9/93	M09	-	S
239	30/8/93	M12	-	S
log	27/10/93	M07	-	S
284	12/10/94	F03	-	RT
	13/10/94	F03	-	RT
	11/11/94	F03	-	RT
297	28/9/93	F06	-	S
log	29/9/93	F06		S
313	12/10/93	M12	-	S
367	10/11/93	F07	-	S
	10/5/94	F07	-	S
382	23/11/93	M14	-	S

* Possum known to have gone up tree, but uncertain if they entered the den.

Den ID	Date	Used by:	Possibly used by:*	Data
384	23/11/93	F03	-	S
log	22/12/93	F03	-	S
	6/11/94	M07	-	RT
390	26/11/93	F02	-	S
392	22/12/93	F03	-	S
log				
397	22/12/93	F02	-	S
log				
473	8/6/94	F03	-	S
488	14/9/94	M07	-	S, RT
	16/9/94	M07	-	RT
	15/10/94	M07	-	RT
	12/11/94	M07	-	RT
	7/12/94	M07	-	RT
493	20/1/93	F01	-	S
	14/9/94	F03	-	RT
	6/11/94	F03	-	RT
	8/11/94	M07	-	RT
	10/11/94	M07	-	RT
	11/11/94	M07	-	RT
	7/12/94	F03	-	RT
	16/12/94	M07	-	RT
	21/12/94	F03	-	RT
494	15/9/94	F03	-	RT
	16/9/94	F03	-	RT
	10/10/94	F03	-	RT
499	11/10/94	F02	-	S
500	11/10/94	F03	-	RT
501	11/10/94	M07	-	RT
log				
505	12/10/94	F02	-	RT
506	13/10/94	M07	-	RT
log				
507	13/10/94	F02	-	RT
508	15/10/94	F03	-	RT
	8/11/94	F03	-	RT
	9/11/94	F03	-	RT
	12/11/94	F03	-	RT
515	8/12/94	M07	-	RT
516	21/12/94	M07	-	RT
517	10/5/94	F02	-	S
	17/8/94	F02	-	RT
	14/9/94	F02	-	RT

Table 34. Use of den sites within individual home ranges by the resident and other animals.

1. Den sites used or possibly used* by resident possum
2. Den sites located in home range but not used by resident possum
3. Den sites used by both the resident and other possums†
4. Den sites used by other possums but not the resident†

Resident	1	2	3	4
M01	24	3	24: F03	3: F03
	99	58	99: M07, F02	58: F01†, M07
	110	73	110: F07	73: F03, M07
		284		284: F03
		13		313: M12
		473		473: F03
		488		488: M07
		501		501: M07
		506		506: M07
M02	29	367	none recorded	367: F02
M04	none recorded	176	NA	176:M09†
		284		284: F03†, M07†
M06	none recorded	313	NA	313: M12
		390		390: F02
M07	58 488	24	58: F01†	24: F03†
	73 493	38	73: F03†	38: F02†
	99* 501	313	99: M01†, F02†	313: M12†
	195* 506	390	239: M12†	390: F02†
	239 515	392	384: F03†	392: F03†
	384 516	494	493: F01, F03†	494: F03†
		499		499: F02†
M08	none recorded	29	NA	29: M02
		367		367: F07†
M09	176	3	none recorded	3: F03†
	237	24		24: M01, F03†
		73		73: F03†, M07
		284		284: F03
		473		473: F03
M12	239	38	239: M07	38: F02†
	313	99		99: M01, M07†, F02†
		390		390: F02†
		499		499: F02
M14	382	none recorded	none recorded	NA
F01	58*	3	58: M07	3: F03
	493	24	493: M07, F03	24: M01†, F03
		73		73: F03, M07
		284		284: F03
		473		473: F03
F02	38 499	239	99: M01†, M07†	239: M07†, M12†
	99 505	313		313: M12†
	390 507			
	397 517			
F03	3 473	58	24: M01	58: F01†, M07†
	24 493	313	73: M07†	313: M12†
	73 494		384: M07†	
	284 500		493: M07†, F01†	
	384 508			
	392			
F06	297	none recorded	none recorded	NA
F07	110*	none recorded	110: M01	NA
	367			

* Animal known to have gone up tree, but uncertain if they entered the den.
† Indicates an animal using a den tree when the resident was alive.

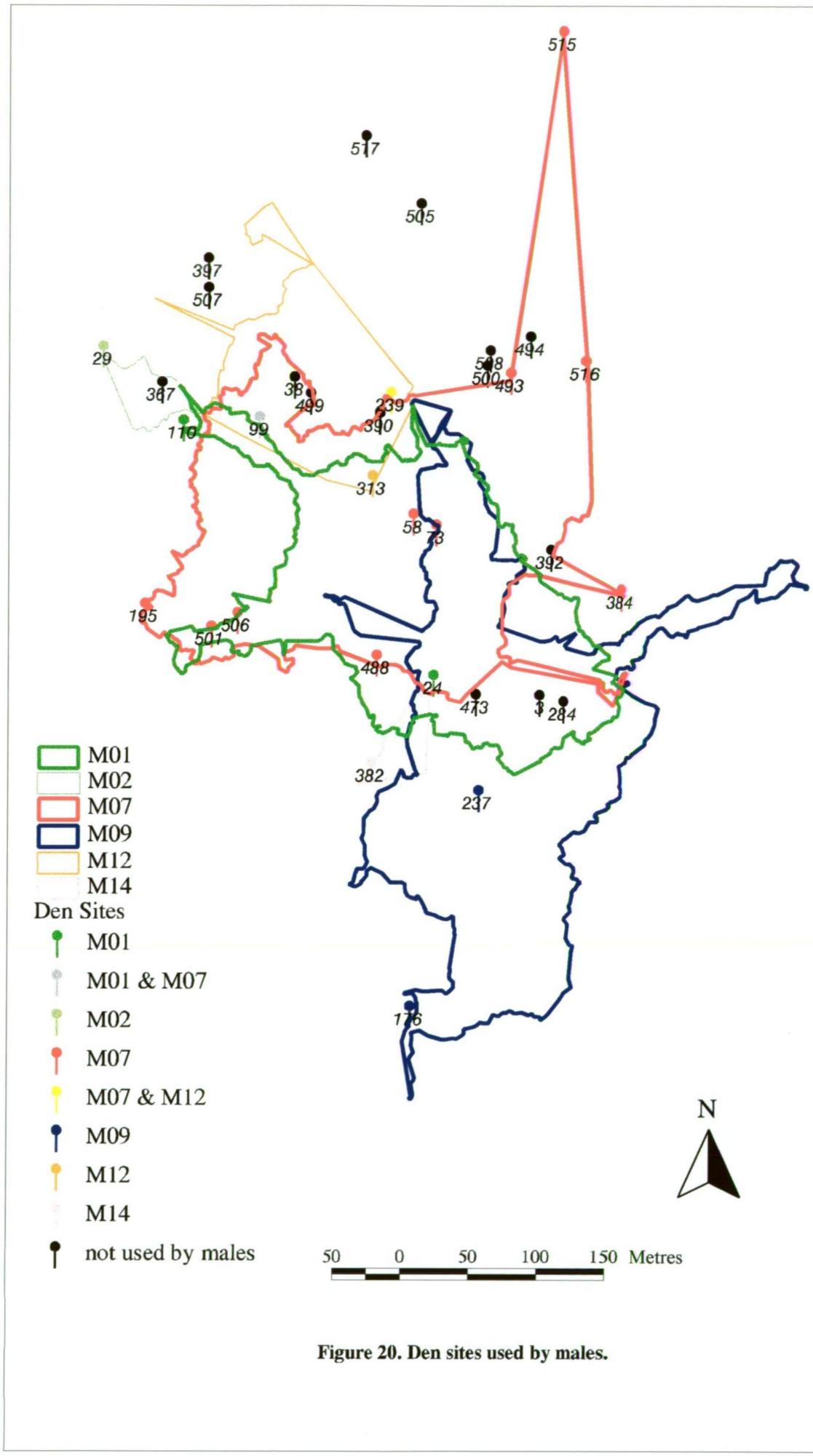

Figure 20. Den sites used by males.

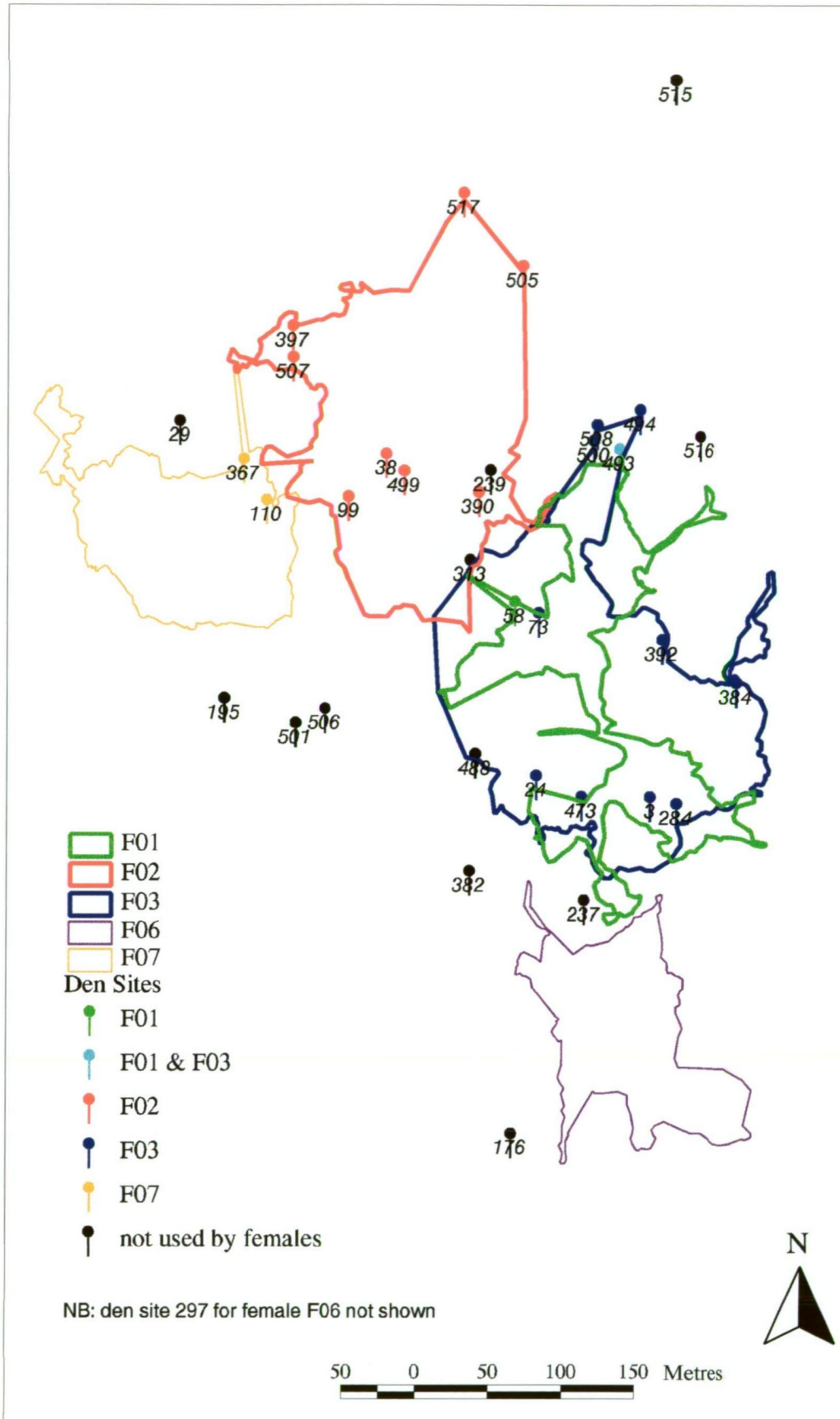

Figure 21. Den sites used by females.

The majority of the den sites were used by one individual only (27/35 = 77%). Eight den sites (numbers 24, 58, 73, 99, 110, 239, 384 & 493) were used by two or three individuals over the duration of the study. Six of these sites (24, 58, 73, 110, 239 & 384) were used by two individuals and in all sites except one (239), one male and one female were recorded using the site. In the sites used by one male and one female there was no overlap in the time periods that the animals occupied the sites, with either the second animal using the site after the disappearance of the first animal (as in den sites 24, 58 & 110) or the second animal using the site more the eight months after the first, with the first animal never recorded as using the site again (as in den sites 73 & 384). The sixth den site used by two individuals was a log (239). Two males used this site within 2 months of one another and the first animal was known to still be using the area at the time the second animal used the den site.

Two den sites (99 & 493) were used by 3 individuals — the first by two males and one female, the second by two females and one male. At both sites there was no overlap in the use of the sites by individuals of the same sex. At den site 99 the first male recorded, M01, had not been captured for two months when the second male, M07, was recorded using the den. Similarly, at den site 493 the first female, F01, had disappeared 13 months before the second female, F03, was recorded at the site. At both these sites, however, the second male occupant (in both cases M07) used the site over the same period as the female recorded at each site (F02 at site 99 and F03 at site 493). At none of the den sites used by more than one animal was more than one individual observed using a den at the same time.

During the radio tracking observations M07 and F03 a number of observations of the use of den sites was revealed. These are shown in Table 35. M07 and F03 were observed to alternate their diurnal use of den tree 493 (highlighted in Table 35). Only occasionally did either possum use a den site for more than one day in a row; each tended to alternate between a number of different den sites.

Table 35. Use of den sites over sequential days by male M07 and female F03.

Date	Den sites used by M07	Den sites used by F03
14/9/94	488	**493**
15/9/94	58	494
16/9/94	488	494
17/9/94	58	3
10/10/94	-	494
11/10/94	501	500
12/10/94	58	284
13/10/94	506	284
15/10/94	488	508
6/11/94	384	**493**
8/11/94	**493**	508
9/11/94	58	508
10/11/94	**493**	-
11/11/94	**493**	284
12/11/94	488	508
7/12/94	488	**493**
8/12/94	515	-
16/12/94	**493**	-
21/12/94	516	**493**

The number of den sites used by an individual varied, although part of this variation is due to methods used to detect den sites. For those individuals for which spool-and-line tracking was the only technique used to locate dens the number of dens is probably underestimated, given that most spool-line ran out before the possum return to a daytime den. This technique revealed between 0 and 3 den sites per possum. For the individuals tracked using radio transmitters (ie M07, F02 and F03) a larger number of den sites were located. Between 8 and 12 den site were located using spool-and-line tracking and radio tracking in these individuals. A similar pattern in the location of den sites within the home ranges of individuals that were radio-tracked can be seen. Den sites for male M07 (see Figure 20), F02 and F03' (see Figure 21) were spread throughout each individuals home range.

The spool-and-line tracking data revealed that den sites were not only used for diurnal denning, but were visited during the night as an individual moved around their home range. Many of the den sites recorded using spool-and-line tracking were used during the night and were not the site that was occupied the next day. Some individuals used more than one den site during the night. For example, female F03 was recorded as using two hollow logs as den sites (384 & 292) in December 1993, and male M07 used den sites 99 and 239 on the same night in October 1993.

4.4. Discussion

Much of the data presented in this chapter confirms findings of other studies about the home range and use of den sites in the brushtail possum. The results of this study are discussed with reference to previous work and provide background information for the following chapter *(Chapter 5. Scent Marking)*.

4.4.1. Home range

Despite the limitations in collecting home range data (as outlined in *§4.2 Methods*) the home ranges in this study were within the ranges previously reported for brushtail possums (see Table 1 in *Chapter 1. Introduction*). Although there is great variation in the size of home ranges reported for different habitats, the results of this study, which was conducted in dry open sclerophyll forest, are comparable to those found in Winter's (1977) study conducted in a similar habitat (ie a modified open grassy forest) in Queensland. The size of the home range appears to be closely related to the type of habitat the possum occupies, the level of resources contained within the habitat and the population density at each site. Hocking (1981) found that possums inhabiting areas of younger regeneration forest, which was a habitat of "lower quality", had larger home ranges than individuals inhabiting older, better quality habitats. A comparison of studies reveals that in areas where the habitat is homogenous home range size does not vary much over the seasons. In more heterogeneous areas there is much seasonal variation in the use of areas within the home range (Hocking 1981), with the availability of food sources influencing home range use (Jolly 1973).

As in all previous reports of home range size (Dunnet 1956; How 1972; Crawley 1973; Jolly 1973; Winter 1977; Hocking 1981; Ward 1984) male home ranges were larger than those of females.

Within each gender there was very little overlap of home ranges in this study. Other studies have reported varying degrees of overlap within gender groups. Dunnet (1956, 1964) found that the individual ranges of resident adult males overlapped to a limited extent. Among adult females there was variation in the degree of overlap between sites. At an "unnatural" site, where possum numbers were higher than in "semi-natural" sites, there was a large amount of overlap in the home ranges of females. There was less overlapping of female home ranges in the lower density, "semi-natural" sites. In New Zealand indigenous forest, intrasexual overlap of home ranges was considerable and there appeared to be no part of a home range reserved for the exclusive use of an individual (Crawley 1973). Crawley suggests that the high degree of overlap may be due to the higher densities of possums at his study site compared to lower densities in other studies where overlap was less. A decrease in home range size with increasing population density has been shown in many mammalian species (Sanderson 1966).

Winter (1977) made a number of important observations about the home ranges of brushtail possums. Males in his study showed considerable overlap in their home ranges. Close examination of the data revealed, however, that there were two classes of male possums. The first group were older (≥4 years old), established males who were dominant to younger males. Among these older males Winter observed that although there was considerable overlap in their home ranges, most of the overlap was due to males moving out of their usual home range to converge on oestrus females during the breeding season. Older established males tended to have ranges with core areas that did not overlap much with adjacent males of similar age. The second group of males were younger (<4 years old), subordinate individuals in the process of establishing a home range. The ranges of these males did not overlap much with other males of a similar age, but often overlapped

extensively with older males. The areas used by younger animals were not as stable as those of older individuals and often changed until they became older and established. Young males were not always successful in establishing themselves in a particular area and Winter believed that success was dependent upon finding a den tree that was not used by an older male or female.

In this study similar groupings among the male possums are evident. Group A in Table 30 were mature individuals caught on a regular basis during the study. There was little home range overlap between these individuals. The adults in Group C in Table 30 have similar characteristics to Winter's younger, subordinate males. Most were caught infrequently and irregularly compared to Group A males and, although only limited information on their home ranges could be collected, it was evident that their ranges overlapped extensively with males in Group A, but not much with each other. Furthermore, despite no significant difference in the body weight, scrotal width, testes length or sternal staining index of Group A and C adults, all these parameters were greater in Group A males. This provides some evidence for an age difference between the two groups. These observations suggest that the Group A males in the current study were older individuals with established home ranges. The Group C adults are most likely younger males in the process of establishing home ranges.

What of the remaining groups of males in this study, that is, the Group B adults and Group C juveniles in Table 30? Winter (1977) and Dunnet (1964) observed that juvenile males are found within the maternal home range until they begin to mature. The two juvenile males captured in this study (ie the Group C juveniles) were both caught without their mothers and can therefore be considered to be independent. Male M02 disappeared from the trapping record during the dispersion period at a body weight that indicated he was close to sexual maturity. The other juvenile, M06, was still a juvenile during the dispersal period. His disappearance from the trapping record in August, while still in a juvenile state, may indicate his lack of success in establishing a permanent home range within the study area. The fate of these individuals is not known; it is possible that both became members of the "transient" group of possums identified by Dunnet (1964) and Crawley (1973). In both studies transient individuals that did not have definite home ranges were captured. These animals were mostly immature and male and were mostly captured between October and January, but particularly during spring. Dunnet (1964) and Crawley (1973) also identified a number of mature males as being transient individuals. It is probable that the mature males belonging to Group C were transient individuals. All five males in this group were capture only once during the study period. Most (4/5) were caught during the dispersion period, the time when juveniles leave the maternal home range. These males were not juveniles, but mature individuals whose body weight, scrotal width, testis length and degree of sternal staining were not significantly different to the resident males that were captured frequently during the study. It is possible that these individuals were transient adults that did not hold resource rich home ranges and that they were passing through the study site in search of unoccupied habitats. It is unlikely that these males were searching for oestrus females to mate with as all of them were caught outside the two recognised breeding periods.

The movements of M07 provide further evidence that mature males move out of their home ranges during the dispersion phase. Between September and December 1994 M07 made a couple of "sorties" into areas outside of his normal home range. Most of these "abnormal" movements were into an area that had previously been occupied by male M09. It is possible that males explore areas adjacent to their normal home range to assess whether a resident male occupies the area.

As well as recognising age-related differences in the home ranges of male possums, Winter (1977) observed that females also showed an age-related structure to their home ranges. Older females (usually >3 years old) tend to have ranges that are exclusive to females of a similar age. Younger females, while not overlapping with other young females, often overlap extensively with older females, particularly their mother. As mentioned previously

there was little overlap between the home ranges of females in this study. In Table 31 two groups of mature females are shown. These individuals have been separated on the basis of the amount of data collected on each, rather than any positive indication that they belong to separate female groups. A comparatively large volume of data was available for the Group A females. These individuals had distinct home ranges that showed little overlap. The age of these females is not known, although the mature female F03 was definitely less than three years old. Fewer data were available for the mature Group B females. Some of these females (eg F06 and F07) appeared to have discrete home ranges with little overlap with the Group A females. For the remaining females there are not enough data available to describe their home ranges. It is possible that some of the Group B females were younger individuals with ranges that overlapped with older females, but this is only supposition.

It is possible, however, to interpret some of the information collected on the juvenile females caught during the study. Among the juveniles there are two groups. The first, Group C in Table 31, were young dependent females caught with their mothers. It is not known what happened to these individuals once they became independent, as they were not trapped at any time after becoming independent. It is possible that they dispersed from the maternal home range. The Group D juveniles, which consists of independent juvenile females trapped without their mothers, contains two types of females. One female was caught only twice over two days during the study. As she was captured in January it is probable that this female was dispersing and just passing through the study area. The other juveniles, females F03 and F08, remained within the study site and became mature. Observations of female F03, who was trapped and spooled frequently, suggest that this individual was female F01's daughter. During the period F01 was trapped F03 was captured within F01's home range. Following the disappearance of F01, female F03 expanded her range to encompass the area previously used by F01.

As in other studies the home ranges of males and females overlapped. Each of the males for which there was good home range data had ranges that overlapped with the home ranges of two females. Dunnet (1964) reported that most males had between 1 and 2 females in their home range, although one male overlapped with between 10 and 11 females. Overlap of home ranges of males and females has also been reported by Crawley (1973) and Winter (1977).

The use of spool-and-line tracking allows a number of important observations about the use of the home range by brushtail possums to be made. Males and females in this study use most of the area contained within the boundary of their home range. Mapping of the individual spool lines also reveals that possums use particular tracks and certain regions in their home range more frequently than other areas. The areas used more frequently are probably analogous to the "core areas" described by Winter (1977). Reasons for more overlap of spool lines in some areas of the home range compared to others was not investigated further in this study, although the spool-and-line technique would enable this to occur. It is probable that these areas are associated with important resources that are required by the possum, such as den trees and feeding trees. Den trees visited often may include those used by the individual as well as those used by other individuals. For example males spend a lot of time during the breeding season visiting the den tree of females within his home range who are in oestrus (Winter 1977). Different feeding trees may be visited regularly during a particular season, for example, when in flower or when fruiting etc. Core areas are a common feature of mammalian home ranges and have been reported in a number of species including phascogales *(Phascogale tapoatafa)* (Soderquist 1995) and red squirrels *(Sciurus vulgaris)* (Wauters & Dhondt 1992).

Spool-and-line tracking reveals that possums cover large areas within their home ranges during one night, often traversing from one end of the range to the other. Hocking (1981) found that during the breeding season, when activity was greatest, individuals could move over at least 50% of their home range between consecutive captures. Winter (1977) observed one male traversing his home range four times during one night. Spool-and-line tracking also provides information on the large distances covered by individuals. The low

number of possums tracked to diurnal den sites because of spool-lines running out indicates that most individuals were travelling more than 1km each night. Other studies have reported various distances being covered by possums during the night. Winter (1977) found that males travel significantly greater distances than females. On average he found that males moved 572m and females 234m, although he recorded one male moving 1630m in one night. A study of possums in a New Zealand forest that bordered with pasture revealed that individuals moved up to 1300m between the forest and pasture in one night (Green & Coleman 1986).

Spool-and-line tracking was a useful technique for monitoring changes in home range used by individuals following the disappearance of neighbouring possums. As described earlier, the juvenile female F03 rapidly expanded into the area previously occupied by female F01. It is interesting to note that other females did not show any evidence of expanding their ranges into the area left vacant by F01. This supports the suggestion that female F03 was the daughter of F01. Due to differences in the dispersal and recruitment patterns of juvenile males and females Clout and Efford (1984) have suggested that neighbouring females are likely to be closely related, whereas males are not. They have suggested that given the longevity of possums in the wild (up to 12 years (Crawley 1970) there is potential for the development of a matrilineal social organisation and the possibility that resources may be shared among related females.

Male possums also showed advantageous expansion of their home ranges. Males M07 and M09 quickly filled the area vacated by male M01, and male M07 continued his range expansion following M09's disappearance. Observations of mammals moving into vacant home ranges or territories has bee reported in many species including red squirrels *(Sciurus vulgaris)* (Wauters *et al* 1995). Evidence that odours play an important role in alerting neighbouring animals that an area has been vacated has been demonstrated in the beaver, *Castor canadensis.* Welsh and Müller-Schwarze (1989) experimentally scented unoccupied beaver sites with a mixture of beaver castoreum and anal gland secretion (ie secretions used by beavers to scent mark their territories). They reported that unoccupied scented sites were colonised less often than unoccupied control sites.

4.4.2. Den sites

Hollows in living and dead trees and hollow logs were used as den sites by possums in this study. In previous studies a variety of sites have been used as dens. In natural habitats in Australia use of holes in the ground, hollow logs, tree stumps and tree hollows have been reported (Dunnet 1956; How 1972; Winter 1977). In New Zealand as well as holes in the ground, hollow logs, and hollows in stumps and trees, possums have been observed to den in thick shrubs (eg gorse), clumps of epiphytes in trees, and fissures in banks and rocks (Kean 1967; Jolly 1973; Green & Coleman 1987; Cowan 1989).

All the species of *Eucalyptus* that were common in the study site were used as den sites. Trees containing hollows in this study had diameters of 50cm or above, and were greater than 14m high. Similar dimensions were reported by Inions *et al* (1989) in an open eucalypt forests in Western Australia. At one site eucalypt trees did not contain hollows large enough for possums until they reached diameters greater than 40cm. At a second site the diameters were greater than 50cm. The mean height of the trees with possum sized hollows was ~19m at both sites. The development of hollows suitable for brushtail possums varies between tree species, even among eucalypts. Mackowski (1984) reported that medium sized hollows appropriate for possums and similar sized arboreal species in Blackbutt trees *(Eucalyptus pilularis)* do not develop until the tree has a diameter between 100 and 120cm.

The majority of den sites used by possums in this study were located off the ground. Spool lines travelling up inside hollow trunks provided evidence that den entrances in the bases of trees led to dens higher up the trunk. A few sites on the ground, all located in logs, were observed in this study. Cowan (1989) reported that den sites on the ground were only used by individuals in poor condition or following periods of heavy rain. Similar observations were made in this study. Both male and female possums were observed to use hollow log as den site during the night at time when it was known to have rained. These sites were used temporarily during the night. Only one male and one female possum were found using den sites located on the ground during the daytime. The female (F06) was observed over two consecutive days using a very exposed den site in a log. She was observed to be in very poor condition and was not trapped again in any subsequent field trips. Male M07 used two hollow logs as diurnal dens over a three-day period. At this time he was also in poor condition and had lost weight. During the two months before these observations each time he was captured he urinated and excreted a thick milky substance from the paracloacal glands. The excretion from the paracloacal glands is believed to be a submissive response in situations of fear (Kean 1967; Biggins 1979). Male M07 had not been observed to react to trapping in this way previously. Two months after being observed denning in the logs male M07 had increased his body weight by 700g (26%) and was using den sites located off the ground during the day.

Reports of studies in Australian forests indicate that the majority of den sites used by possums are located above the ground. In New Zealand forests the percentage of above ground dens varies greatly between studies. Green and Coleman (1987) reported that only 25% of dens in a mixed hardwood forest were located above the ground and that most of these were only slightly elevated (eg mean height in living trees: ♂ = 4.4m, ♀ = 7.4m. mean height in dead trees: ♂ = 2.6m, ♀ = 2.0m). The remaining 75% of dens were at or below ground level or amongst root system in living trees, or in the root systems of dead stems or logs. In a podocarp and mixed hardwood forest in New Zealand Cowan (1989) observed that 98% of dens were above the ground in trees, particularly among clumps of epiphytes. The remaining dens were either under fallen log or trees, in dense tangles of gorse, at the bases of trees or underground in rotted-out tree stumps.

More than three-quarters of the dens identified in this study were used by one individual only, with the remainder being used by two or three individuals. A similar result was reported by How (1972). He observed that 65% of dens were used by one possum, 23% were used by two individuals and the rest were used by 3-4 individuals. Similarly, Cowan (1989) found that 50% of dens were used by one individual, although he observed that dens were often used sequentially by several, and up to 9, different possums. Over three years Winter (1977) found that between 38% and 56% of dens were used by one adult possum in a 12-month period.

Sharing of dens by individuals of the same sex is rare. Cowan (1989) found that 60% of dens used more than once by a female and 65% of dens used more than once by a male were not used by other possums of the same sex. In the current study there was only one instance of a den site being used by two individuals of the same sex (ie males M07 and M12) when both were known to be alive and using the area. The den site was a log used by both individuals during different nights, probably to shelter from the rain. The site was not used as a diurnal den by either male. In all other records of same sex individuals using a den site, the first possum was known to have disappeared from the site before the second was observed to use the den. The lack of dens been used by more than one individual of the same sex during the same time period is a reflection of the low level of home range overlap between same sex individuals at this site.

Some sharing of dens between individuals of the opposite sex was evident, although no sharing of a den during the same day or night was recorded. Sharing of dens during the same day has been reported in other studies, although it was not a common occurrence. Adult possums rarely shared dens in Winter's (1977) study; 89.5% of observations were of

solitary use by an adult male or an adult female with or without a joey. Winter did observe adult males and females sharing a den on nine occasions — all of these observations took place during the consort period prior to breeding and involved established individuals. One young adult male was recorded sharing a den with his mother. Cowen (1989) also reported that den sharing was uncommon. Indeed most of the small number (3.4%) of observations he made of two and sometimes three individuals in a den tree were actually individuals using separate dens located in the same tree. The majority (75%) of the pairs of possums found in the same tree consisted of a male and a female. The remainder were pairs of males (3%) and pairs of females (22%). Dunnet (1956) observed that dens were used by more than one individual, but did not record more than one possum using a site at the same time. Crawley (1973) on the other hand reported that diurnal den sites were often shared by several animals. It should be noted that the home ranges of individuals in Crawley's study were comparatively small and the densities of possums higher than those reported for other studies.

Use of spool-and-line tracking reveals that den sites are used in at least two different ways by possums. Dens in hollow logs and dens in trees were often used during the night by possums — in some cases this was probably to shelter from inclement weather. In the day diurnal den sites were mainly found in trees, with sites on the ground being used by sick or injured individuals. Other mammalian species show a similar pattern of den use. The diurnal red squirrel *(Sciurus vulgaris)*, for example, uses "resting nests" during the day and "sleeping nests" during the night (Wauters & Dhondt 1990). Some sites were only used during the day for resting; others were used for resting and sleeping, while most were used only during the night as sleeping nests.

The radio-tracking observations of male M07 and female F03 illustrate the pattern of den use and den sharing by possums with overlapping home ranges. Male M07 used eight and female F03 used six different diurnal den sites over a 4-month period. Only one of these sites, #493, was used by both individuals during this period, although there were seven dens sites (numbers 24, 58, 73, 384, 392, 493 & 494) found within the overlap of the two home ranges. The use of site 493 alternated between the two possums. Neither possum was observed to spend more than two consecutive days in this den or any other den. During the whole study period female F03 used all sites contained in the area of overlap, except site #58. This site was used comparatively often (4/28 observations) by male M07. Male M07 used only 4 of the den sites (ie 58, 73, 384 & 493) in the area of his range that overlapped with female F03; he was not observed to use dens 24, 392 or 494. Female F03 used these sites (ie 24, 392 & 494) in 6/25 observations. Cowan (1989) reported that possums changed den sites on average two nights out of three. He also observed that in 16% of observations a den site was occupied by different possums on consecutive days. Winter (1977) found that females changed den sites less frequently than males and often used the same den for several months.

Among the individuals with adequate data (ie those observed using radio-tracking and spool-and-line tracking) between 8 and 12 den sites were recorded for each. In other studies the number of dens sites per individual varies. Over a twelve-month period Green and Coleman (1987) observed individuals using between 10 and 15 den sites, with immature possums using less sites than mature possums. Cowan (1989) reported that individual possums used between 11 and 15 den sites over a 12 month period. Most, however, were only used occasionally, with the three most commonly used accounting for 60-75% of observations. Cowan observed that a few possums used many more dens than the average number — these individuals included juveniles born in the study area, adult males observed soon after arriving on the study site and an adult female who shifted range during the study. Possums followed to dens by How (1972) used between 1 and 13 dens; most individuals followed more than once used more than one den site. Observations of 6 central animals in Winter's (1977) study revealed that between 2 and 5 dens were used. Dunnet (1956) also reported that possums use more than one den. Winter (1977) and Cowan (1989) both reported that males tended to use more dens than females.

Dens in this study appeared by be spread throughout the home ranges of possums of both sexes. A similar pattern was observed by How (1972).

The results of this study are supported by previous work on the brushtail possum. In the following chapter on sternal gland scent marking data presented in this chapter provide background information.

Chapter 5. Scent Marking

5.1. Introduction

Secretions from sternal integument of the brushtail possum, *Trichosurus vulpecula,* are deposited in the environment using a behaviour variously known as "sternal rubbing", "chesting" or "sternal sliding"(Bolliger & Hardy 1944; Winter 1977; Biggins 1979). The possum adopts a bent leg stance and lifts its chin away from the substrate enabling the chest to come into contact with the object being marked. Using a forward motion the chest is rubbed up the substrate and then lifted off. This action may be repeated a number of times (Winter 1977). Winter (1977) observed that chesting by females was less vigorous than in males and usually consisted of a single, light rub. Plate 8 shows a captive male possum using the sternal gland to mark his enclosure.

Observations of scent marking and olfactory communication have been studied in the brushtail possum in field and captive situations. In an extensive field study, conducted in an area of modified open grassy forest in Queensland by Winter (1977), the importance of scent marking and olfaction was investigated as part of a study on the behaviour and social organisation of the possum. Studies of scent marking in captive possums have also been conducted, the best known being a study of olfactory communication in male possums by Biggins (1979). From these studies a range of important observations about sternal scent marking in the brushtail possum have been made. They include information about the types of objects marked, the location of objects in the home range, temporal (nightly and seasonal) scent marking patterns, maturity, gender and hierarchical differences in marking behaviour.

Winter (1977) observed that possums use the sternal integument to mark tree trunks, branches, fallen logs, bare earth and tufts of grass. Chesting behaviour was first observed in juvenile females as young as 8 ½ months old. The behaviour in males was not observed until the age of 21 months, although this may have been due to lack of observations of juvenile males (Winter 1977). It was noted that no juvenile males were observed to use the sternal gland to mark while they remained in the maternal home range.

Among adult male possums Winter (1977) observed temporal patterns in sternal marking behaviour. There was a marked peak early in the evening with most marks being made on the bases of trees. A smaller peak occurred at dawn and consisted mainly of marks on the branches of a den tree as the animal returned to its den. Seasonal patterns in chesting among males were also evident. A distinct peak was seen in March, with other peaks in June and October. Between October and December little sternal marking was observed in males. Among female possums most scent marking (~77%) was done between the first and last time a joey was seen riding on the mother's back.

Sternal rubbing may be associated with another form of scent marking known as "chinning" or "labial sliding". Secretions from integumentary glands on the chin, from labial gland on the upper and lower lips and salivary glands are deposited on objects using this behaviour. Although chesting and chinning are separate and easily distinguished behaviours, on occasion they may be performed together. Chinning may be followed by a chesting movement, or may be part of a chinning-chesting combination where an individual continues a chesting movement by wiping his chin and the side of his mouth against the substrate. Winter (1977) observed that chinning and chesting (performed

separately and together) were the most common forms of scent marking in the field and that most of these marks (91%) were made by males. Chesting and chinning are performed in a number of contexts. For males there were three main contexts: scent marking with no other possum in the vicinity, scent marking with an oestrus female in the area and scent marking at dens sites at dusk and dawn (Winter 1977). Biggins (1979) reported that sternal gland marking among captive male possums was more common in dominant individuals than subordinates. Dominant males placed in novel or odour-marked cages displayed higher levels of sternal marking than subordinates and in agonistic encounters between males sternal rubbing was the most common form of scent marking.

Field observations of females showed, that like males, chesting and chinning are used to mark the den when leaving at dusk and when returning at dawn, although females were not observed to mark their dens as often as males (Winter 1977). Other contexts in which female possums were observed to mark included scent marking without other possums in the vicinity, scent marking with a joey following and scent marking at den sites of other individuals (Winter 1977). Winter observed that although marking by males and females was performed without another possum in the vicinity, a high proportion of marking did occur in the vicinity of another possum or in areas known to be used by others.

No evidence of boundary marking by male possums was observed in Winter's study. Scent marks did not appear to deter or prevent other males from entering the home range of the marker. Chesting and chinning were spread throughout the home range, but appeared to be concentrated in definite areas, particularly den trees that were used regularly or visited by the resident male or other males and trees used for feeding.

In observations of the response of possums to scent marks Winter (1977) reported that most individuals showed a low level of reaction to sternal and chin marks, often ignoring them completely. Adult females in particular showed complete lack of interest in male scent marks. There was some evidence that adult male marked over marks made by another male, but Winter did not observe males marking over their own marks.

The aim of this study is to investigate gender and seasonal differences in sternal gland scent marking highlighted in previous studies. This study has focused on collecting data on scent marking in a natural population of possums using a novel technique combining spool-and-line tracking and fluorescent pigments (see *Chapter 3 Recording Scent Marking in the Field).* Sternal gland scent marking data collected using these techniques will be presented. Other information related to the sternal gland and sternal gland scent marking, including a range of data not collected in previous studies, will also be presented.

Plate 8. Captive male possum "sternal rubbing" his enclosure.
(Photo: K. Hynes)

5.2. Methods

Sternal gland scent marking was investigated in a field study of brushtail possums undertaken in the Mount Morrison State Forest in Tasmania. Details about the study site and the field methods used (ie trapping, handling and measurement of animals, and measurement of the home range and den sites) are given in *§4.2 Methods* of *Chapter 4 Home Range and Den Sites.* The main aim of the field study was examine gender and seasonal differences in sternal scent marking in the brushtail possum. For males comparisons of marking over the four seasons of the year (ie pre-breeding, breeding, post-breeding and dispersal) were made. For females differences in the location of scent marks were examined on the basis of the reproductive state of the individual (ie immature, anoestrus, oestrus and with young), although the seasons were used in some of the analyses. Definitions for the four seasons and the reproductive states of females are given in *§4.2.2.2 Handling and measurement.* Methods used specifically in this part of the study outlined below.

5.2.1. Sternal scent marking of home range

Sternal gland scent marking was recorded between November 1992 and December 1994. A detailed explanation of the methods used to locate sternal gland scent marks is given in *Chapter 3 Recording Scent Marking in the Field.* Briefly, a combination of spool-and-line tracking devices and fluorescent pigments were used. Possums were lived trapped, sedated and fitted with either 3- or 4-reel spool-and line-devices and had a smear of pigment mixture applied to their sternal region. The end of the spool-and-line device was tied to the trap that the animal was caught in. Animals were left unrestrained in hessian sacks to recover at the point of capture. This enabled them to leave when they had fully recovered without further human disturbance. The resulting trailing spool line was followed the next day and any sternal gland scent marks were noted. A total of 99 successful spool-and-line observations were made (56 male and 43 female) using 14 individuals (8 males and 6 females).

For each scent mark the type of substrate on which it was located and the size of the pigment smear (length and width) was recorded. The locations of the scent marks in the study site were recorded using GPS and mapped using *ArcView* (Environmental Systems Research Institute (ESRI), Inc.). When a mark was found on a tree, the species, the height and DBH of the tree, and the height of the mark from the ground were also recorded. The relationship of scent marks to den sites and feeding trees was recorded. Using spool-and-line tracking it was possible to classify the trees used by possums. Den trees were identified as those in which spool-lines were observed to go up and into, or possibly into, den entrances. A tree was classified as a probable feeding tree when spool-line was observed to go up the tree and out along branches to areas of foliage.

A rate of sternal gland scent marking for each individual was determined by calculating the number of scent marks made per 100 metres of spool-line for each collection date. When an individual had more than one spool in a month an average rate was calculated using all the spool observations for that month.

Patterns of scent marking were explored using *ArcView* and *ARC/INFO* (ESRI, Inc.). The distance of scent marks from the marker's home range boundary was calculated. The purpose of this was to determine whether there was any boundary or hinterland pattern in the position of sternal scent marks in a possum's home range. It is extremely difficult to determine an accurate and absolute home range boundary for an individual at any point in time. This is because the outer limit determined by spool-and-line tracking and trapping

data may not be a true representation of the home range boundary of an individual (see *Chapter 4 Methods* for specific difficulties in estimating home range boundaries in this study). Furthermore, even though home ranges relatively stable in the brushtail possum the boundaries are dynamic and may be influenced by changes in the possum population and seasonal changes. There was no accurate, objective way of determining the boundaries and therefore whether or not scent marks were located on the boundary or not. To examine the possibility of boundary or hinterland scent marking in the possum the distance of each scent mark from a probable home range boundary was calculated. Scent marks were grouped into one of five categories (ie < 5m, 5.0-9.9m, 10-14.9m, 15.19.9m and ≥ 20m) based on their distance from the edge of the home range.

5.2.2. Marking of den sites

Between September and December 1994 four possums were fitted with collar-style radio transmitters (Sirtrack, New Zealand) to facilitate the location of den sites and enable observations of behaviour at den sites to be made. Two males (M7 and M12) and two females (F2 and F3) were chosen. During the day each collared animal was located using a handheld Yagi antenna and a receiver. One animal was selected for observation per night. A total of fifteen observations of behaviour at den sites were made (eight observations of males and seven observations of females). The behaviour of animals was observed for the time they emerged from the den until they either: (1) returned to the den and did not re-emerge for more the 30 minutes; (2) left the den tree by moving to another tree or to the ground; or (3) remained in the den tree without actively moving around for more than 30 minutes. A record of the movement patterns, grooming, sniffing, scent marking (sternal rubbing, chin wiping, combined sternal and chin rubs, cloacal dabbing), and urination were made.

Unfortunately two of the collared animals died soon after this section of the work began. Male M12 was found dead three days after having the collared fitted. His body was located in the private property adjacent to the study site. It is believed that he died of 1080 poisoning as this pest control substance had been laid around the plantation on the property approximately two days earlier. There was no evidence to suggest that the collar had in any way resulted in the death of this animal. No den site observations were made on this animal. Female F02 was also found dead after being collared. It is likely that the 1080 also poisoned this animal. Again there was no evidence that the collar had anything to do with the death of this animal. One observation at a den site was made before this animal died.

5.2.3. Sternal staining index

As part of the routine data collection in the field each time an individual was trapped, the length and width of the stained fur surrounding the sternal integument was measured. The same information was collected from roadkill possums discussed in *Chapter 2. Histology of the Sternal Integument.*

To examine changes in the size of the staining a "sternal staining index" was calculated by multiplying the length of the stained area by the width. Stoddart (1980c) used a similar method to create an index of activity for the subauricular gland in bandicoot species. Seasonal changes and differences between mature male and female field and roadkill possum were investigated. For the field animals an average "sternal staining index" was calculated for each individual for each season. For the roadkill animals an average was calculated for all mature males and all mature females collected in each season.
A one way ANOVA between the seasonal groups was performed for the roadkill data.

The sternal staining index of the female field and roadkill animals was also examined using reproductive status. The following categories: immature, anoestrus, oestrus and with young were used. (Note: In some cases it was difficult to categorise the roadkill females. See *Chapter 2. Histology of the Sternal Integument* for a discussion the difficulties and their implications.). A one way ANOVA was performed on these data sets.

5.3. Results

5.3.1. Description of scent marks

A total of 169 scent marks were recorded between November 1992 and December 1994 using spool-and-line tracking and fluorescent pigments. One-hundred-and-twenty marks were made by males (n=8) and 49 by females (n=6). A crude calculation taking into account the number of spool-line followed, but without considering the total distances covered by males and females (this is done in Figures 22 & 23), shows that males marked almost twice as often as females. Over 56 spool lines males made 120 scent marks, or 2.1 marks per spool; over 43 spool-lines females marked 49 times, or 1.1 marks per spool-line.

Scent marks were made on a variety of objects: tree trunks, branches of shrubs, clumps of grasses, fallen logs, fallen sticks, branches and bark on the ground, pieces of wood (from past logging activities), rocks, and on objects (ie. rock, bark etc.) used to cover traps. Table 36 lists the range of objects and the frequency with which they were marked. Plates 9, 10 and 11 show scent marks on some of the objects marked.

Table 36. Type and frequency of objects marked using the sternal gland.

	♂♂			♀♀		
Object marked	No. scent marks	No. of scent marks on or within 2m of: Tree	Trap	No. scent marks	No. of scent marks on or within 2m of: Tree	Trap
Tree trunk	27	27	-	6	6	-
Pieces of wood & fallen sticks/branches/bark	65	10	14	27	-	4
Rocks	13	2	3	12	2	5
Logs	14	4	2	1	-	-
Shrubs & ground covers	-	-	-	3	-	-
Traps	1	-	1	-	-	-
Total	120	43 35.8%	20 16.7%	49	8 16.3%	9 18.4%

Although a variety of objects were marked, many of the marks, particularly those made by males, were made close to trees and traps. Males marked more often on trees and traps or on objects within 2m of trees and traps than females. Just over half (52.5%) of males scent marks were associated with trees or traps; only 34.7% of female scent marks were associated with trees or traps. The majority (65.2%) of female scent marks were made on objects without any close association with a tree or a trap.

Plate 9. Scent mark on a rock.
Scale: large divisions on tape measure = 1cm
small divisions = 1mm
(Photo: K. Hynes)

Plate 10. Scent mark on pieces of wood.
Scale: large divisions on tape measure = 1cm
small divisions = 1mm
(Photo: K. Hynes)

Plate 11. Scent mark on a tree trunk.
Scale: large divisions on tape measure = 1cm
small divisions = 1mm
(Photo: K. Hynes)

Table 37. Length, width and index* of sternal gland scent marks (mean ± SD and range) and height of scent mark from ground (where applicable).

	All scent marks	Scent marks on trees	Scent marks on objects other than trees
♂♂	n=120	n=27	n=93
Length (cm)	5.9 ± 4.5 (0.5-30.0)	9.3 ± 4.4 (1.5-18.0)	4.9 ± 4.0 (0.5-30.0)
Width (cm)	2.1 ± 2.1 (0.1-14.0)	3.1 ± 3.0 (0.5-14.0)	1.8 ± 1.6 (0.3-6.0)
Index (length x width)	16.9 ± 28.9 (0.1-156.0)	34.3 ± 47.0 (1.0-156.0)	11.2 ± 18.0 (0.1-135.0)
Height off ground (cm)	30.3 ± 16.3 (10.0-74.0) n=37	31.7 ± 17.4 (0.0-74.0) n=27	19.4 ± 13.9 (10.0-54.0) n=10
♀♀	n=49	n=6	n=43
Length (cm)	4.1 ± 3.6 (0.5-17.0)	9.4 ± 5.9 (3.0-17.0)	3.5 ± 2.7 (0.5-12.5)
Width (cm)	1.6 ± 1.0 (0.5-6.0)	3.0 ± 1.8 (2.0-6.0)	1.4 ± 0.7 (0.5-3.0)
Index (length x width)	8.7 ± 13.8 (0.5-84.0)	32.8 ± 32.0 (4.5-84.0)	5.7 ± 5.6 (0.4-18.8)
Height off ground (cm)	70.3 ± 89.3 (17.0-255.0) n=8	88.0 ± 102.9 (17.0-255.0) n=6	26.0 ± 9.9 (19.0-33.0) n=2
Both sexes	n=169	n=33	n=136
Length (cm)	5.4 ± 4.3 (0.5-30.0)	9.3 ± 4.5 (1.5-18.0)	4.5 ± 3.7 (0.5-30.0)
Width (cm)	2.0 ± 1.8 (0.1-14.0)	3.1 ± 2.8 (0.5-14.0)	1.7 ± 1.4 (0.5-30.0)
Index (length x width)	14.3 ± 25.7 (0.1-156.0)	34.1 ± 44.6 (1.0-156.0)	9.5 ± 15.4 (0.1-135.0)
Height off ground (cm)	37.1 ± 40.6 (10.0-255.0) n=45	40.5 ± 45.3 (0.0-255.0) n=33	20.6 ± 13.1 (10.0-54.0) n=12

* The sternal scent mark index is the product of the length and width of the scent mark.

Variation in the size of scent marks is shown in Table 37. The size of scent marks made by male possums on objects other than trees were larger than those made by females. There was no gender difference in the size of scent marks made on trees. For both sexes the marks made on trees were larger than those made on other objects.

The height of scent marks made on trees were, on average, less than one metre from the ground. Indeed, only two scent marks were made above a height of 75cm; both these marks were made by female possums while climbing up the tree. It should be noted, however, that any scent marks made higher up (greater than approximately 5m) on trunks or among branches that could not be seen from the ground may have been missed. It was not possible to check for scent marks when spool-lines went up into trees. In *§5.3.5 Marking of den sites,* some data on scent marking in trees is presented.

Table 38. Characteristics of trees associated with scent marking: species, tree height, DBH and height of nest entrance (range, mean ± SD).

	♂♂		♀♀	
Species	Tree Height (m) (L= height of living foliage; D= height including dieback)	DBH (cm)	Tree Height (m) (L= height of living foliage; D= height including dieback)	DBH (cm)
E. pulchella ♂♂ (n=8) ♀♀ (n=2)	L (n=8): (7.9-29.7)16.8 ± 7.5 D (n=3): (20.1-33.7)26.5 ± 6.8	(10.9-125.5) 68.9±40.2	L (n=2): (8.9-12.1)10.5 ± 2.3 D (n=1): 12.65	(10-72.9) 41.5±44.5
E. viminalis ♂♂ (n=8) ♀♀ (n=3)	L (n=8): (12.5-25.7)19.1 ± 3.9	(21.6-78.6) 48.4 ± 20.0	L (n=3): (16.1-24.7)19.5± 4.5 D (n=1): 31.3	(32.4-73.1) 49.8 ± 21.0
E. amygdalina ♂♂ (n=8) ♀♀ (n=1)	L (n=8): (10.3-33.7)23.8 ± 7.6 D (n=2): (17.7-29.7)23.7 ± 8.5	(39.5-122.9) 73.4 ± 31.6	L (n=1): 20.1 D (n=1): 21.7	37.0
E. obliqua ♂♂ (n=3) ♀♀ (n=0)	L (n=3): (8.7-34.9)18.1 ± 14.6	(10.2-62.6) 28.3 ± 29.7	-	-
Dead ♂♂ (n=1) ♀♀ (n=0) species unknown	D (n=1): 14.7	33.4	-	-
Juvenile *Euc.*spp. ♂♂ (n=43) ♀♀ (n=1)	-	-	L (n=1): 1.7	1.4
Acacia mearnsii ♂♂ (n=1) ♀♀ (n=0)	L (n=1): 8.7	17.0	-	-
ALL TREES ♂♂ (n=29) ♀♀ (n=7)	L (n=29): (7.9-33.7) 19.3 ± 7.8 D (n=6): (14.7-33.7)23.6 ± 7.4	(10.2-125.5) 57.3 ± 33.5	L (n=7): (1.7-24.7) 14.5 ± 7.6 D (n=3): (12.7-31.3)21.9 ± 9.3	(1.4-73.1) 38.7±27.8

The characteristics of trees associated with scent marking (ie trees that were marked directly or had marks made within two metres of their bases) are given in Figure 38. Note: The number of trees does not equal the number of scent marks for two reasons. Firstly, on a number of occasions more than one scent mark was made on a tree, or on objects within two metres of a tree, by the marker at a particular time. Secondly some trees were marked on more than one occasion by the same or a different individual (for more details see *§5.3.2 Over-marking).*

Male possums made 43 scent marks on or within 2 metres of 29 different trees. The path of the spool-line indicated that 67.4% of the scent marks were made as the possum walked past the tree. In 20.9% of cases the possum climbed up the tree and in 9.3% the possum used the hollow base of the tree as a den site during the night. In the remaining 2.3% of cases it is not known what the animal did as the scent mark was located after the spool line had finished.

Of the 29 trees marked by males only one third (n=9) had a record of possums climbing up them: 4 had been climbed by the marker immediately after he marked, 2 were climbed by the marker on a date other than when he marked, and 3 were climbed by another possum. Almost two-thirds of the trees that were marked or had marks within two metres of their bases were never knowingly climbed. There does not appear to be any seasonal distribution of marks made on or near trees and whether or not the tree was climbed or passed by. In all four seasons there were instances of male possums marking and climbing trees, and marking and passing by trees. Two of the twenty-nine trees associated with male scent marks were known den sites. Both trees (numbers 73 and 58) were marked by Male M07, on 29/6/94 and 14/9/94 respectively. Neither tree was marked on the trunk; male M07 marked on a rock and a stick next to tree 73 and on two pieces of wood next to tree 58. M07 was known to use a den in both trees. The den in tree 73 was situated in the hollow base of the tree and was used by M07 on two occasions as a den during the night. Female F03 also used this den in this tree during the night on one occasion. The den in tree 58 was located above the ground. Male M07 climbed up tree 58 after marking on the wood below. The spool-line did not come back down this tree and radio-tracking revealed that M07 was using the tree as a diurnal den site. M07 was radio-tracked to this site during the day on a number of occasions (see Table 33, *Chapter 4 Home Range and Den Sites).* Female F01 was recorded in tree 58 in February 1993 and possibly used the den. She was not recorded to scent mark at this time and had disappeared from the study area by the time M07 was recorded as using the tree.

For females, 57.1% of the scent marks were made as the possums walked past the tree and in the remaining 42.9% the possums marked and climbed up the tree. In one case it is highly probable that the juvenile *Eucalyptus* sp female possum F01 marked and climbed up was used as a food source — there was evidence that a number of leaves had recently been eaten.

Of the seven trees marked by females only one, tree 110, was climbed by another possum. This tree was also the only den site observed to be associated with female sternal scent marking. On the 28/9/93 while climbing up tree 110 female F07 made a sternal scent mark on the trunk. It is highly likely that female F07 climbed up to enter a den in the tree. At this time F07 was carrying a 3-4 month old pouch young. Female F07 possibly used this tree again as a night-time den in March 1994. This time female F07 was in anoestrus and did not mark the tree as she climbed toward the den. This tree was climbed and used as den site by male M01 who had disappeared from the study site in May 1993, a month before female F07 was recorded in the area.

Information on the marking of diurnal den sites obtained by direct observation is given in the *§5.3.5 Marking of den sites.*

5.3.2. Over-marking

Over-marking was a rare event (see Tables 39 and 40 below). Six instances of an individual marking over or immediately adjacent to a mark they had previously made were recorded. Three instances of more than one individual marking in the same location were recorded. Males and females were both observed to mark over their own scent marks. Both sexes also marked over marks made by other individuals. Although the sample size is small the most common over-mark of this type was a male marking over the mark of another male. There was only one instance of a female over-marking were a male had scent marked, and one of a male marking were a female had marked. Plate 12 shows the scent marks made on a piece of wood by M01 (orange) and F01 (pink) in Table 42.

The time interval between marks by the same individual on an object ranged from 3 days to 17 months. The time interval between marks on an object made by different individuals ranged from 1 to 17 months. These intervals should be treated cautiously as only a small percentage of the total number of sternal scent marks made by possums at this site could be recorded. It is probable that some over-marking was missed and that the average time interval between marks is less than the maximum times given above.

It should also be noted that in some of the over-marks by the second individual were made on objects after the original marker had disappeared from the site. Male M09, for example, marked on the pieces of wood 7 months after F01 and 10 months after M01 had disappeared. Similarly, M07 marked tree 91 sixteen months after male M01 had disappeared.

Table 39. Over-marking by the same individual.

Animal	Date	Season	Object marked	Scent mark index (cm^2)	Height off ground (cm)*
F02	31/5/93	Breeding	Stick	9	-
	12/10/94	Dispersal		16	-
M01	5/4/93	Breeding	Tree 48	30	13
	20/4/93	Breeding	*E. amygdalina*	3	18
M07	28/9/93	Post-breeding	Bark at base of Tree 288	2	-
	13/10/93	Dispersal	Wood at base Tree 288 *E. pulchella*	2	-
M09	8/2/94	Breeding	Tree 428	24	25
	8/3/94	Pre-breeding	*E. amygdalina*	25	37
M09	12/4/94	Breeding	Stick	0.5	-
	15/4/94	Breeding		5	-
M09	20/12/93	Dispersal	Stick	7.5	-
	8/3/94	Pre-breeding		10.5	-

* Height of scent mark from the ground measured from the bottom of the scent mark.

Table 40. Over-marking by different individuals.

Object marked	Animals	Date	Season	Scent mark index (cm^2)	Height off ground (cm)*
Piece of wood	M01	20/4/93	Breeding	8	-
	F01	17/6/93	Breeding	19	-
	M09	8/3/94	Pre-breeding	12 & 22	
Tree 49	M04	15/9/93	Post-breeding	14	74
	M09	27/10/93	Dispersion	12	62
Tree 91	M01	5/4/93	Breeding	30	30
	M07	14/9/94	Post-breeding	12	25

* Height of scent mark from the ground measured from the bottom of the scent mark.

Plate 12. Over-mark on a piece of wood.

M01 20/4/93 orange (on left of picture), F01 17/6/93 pink (very faint on right of picture, circled).
M09 marked on bits of wood next to these on 8/3/94 (after the photo was taken).

Scale: large divisions on tape measure = 1cm
small divisions = 1mm
(Photo: K. Hynes)

5.3.3. Sternal scent marking rate by gender, season and reproductive status

Figures 22 and 23 show the rate of scent marking for males and females, respectively, over the duration of the field study.[2] Figure 24 provides information on the rate of scent marking for individual females based on their reproductive state.

Figure 22 includes all the mature males belong to Group A of Table 30 in Chapter 4. These were the males considered to be older males with established home ranges. There were no data available for the Group B males, ie the transient adult males, as these individuals were only caught once and were not tracked using spool-and-line devices. The other mature males captured at the site, the Group C males, were caught infrequently and were considered to be younger and subordinate to the Group A males. Only one of these males, M14, was tracked using spool-and-line. He was not observed to mark using his sternal gland. Similarly one juvenile male, M02, was tracked during the study; he was not observed to mark.

Examination of the marking data reveals that mature males which have established home ranges have very low rates of marking during the period when females at the site were carrying young. At the end of the post-breeding period, at the time of secondary breeding, there is an increase in marking,which carries through the dispersal phase and into the pre-breeding period.

Figures 23 and 24 contain data for most of the mature females classified in Groups A and B of Table 31 in Chapter 4. There are no data available for females F05 and F10 as neither animal was tracked using the spool-and-line technique. Examination of the females during the year in Figure 23 reveals that the majority of the sternal marking performed by mature females was done during the time when they had young in the pouch or dependent young on their backs. Most of the remaining marking occurred during the dispersal period. Because not all females had young during the breeding season and through the post-breeding period, it is useful to consider the rate of marking of individual females in different reproductive states. The results shown in Figure 24 reveal that the highest rates of sternal marking are indeed seen in females with young. A lower level of marking occurs in mature females in oestrus.

Most of the spool-and-line observations were of mature animals (see Table 29 in *Chapter 4)*. A small amount of information, however, was collected on two immature individuals. Male M02 was tracked twice during the dispersal phase in 1992; no scent marking was observed (Note: these data are not shown in Table 29). Female F03 was tracked three times (between January and early November 1993) while immature. During this time no scent marking was observed. Between late November 1993 and March 1994 female F03 began to mature, showing an increase in body weight, pouch development and increased staining across the sternal region. During this period female F03 was tracked twice and was observed to scent mark during the dispersal months of November and December (see Figure 24).

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[2] NOTE: There are six spool-lines that are not included in the Figure 22, 23 and 24. These are spools where information was collected over a distance of less than 50m. There are two problems associated with these spools. Firstly, the short distance may results in scent marking that occurred later in the night being missed. Secondly, when scent marking did occur in the first 50m of spool the method of calculating the number of scent marks per 100m tends to results in a gross over-estimation of the rate of marking. For these reasons the following spools have not been included in the main graphs.

F01	Aug '93	24m	0 Scent marks	F07	Oct '93	19.5m	2 Scent marks
F02	May '94	45m	0 Scent marks	F07	Nov '93	14.5m	0 Scent marks
F06	Sept '93	27m	4 Scent marks	M07	Jun '94	49m	7 Scent marks

Figure 22. Scent marks per 100m of spool (Males)

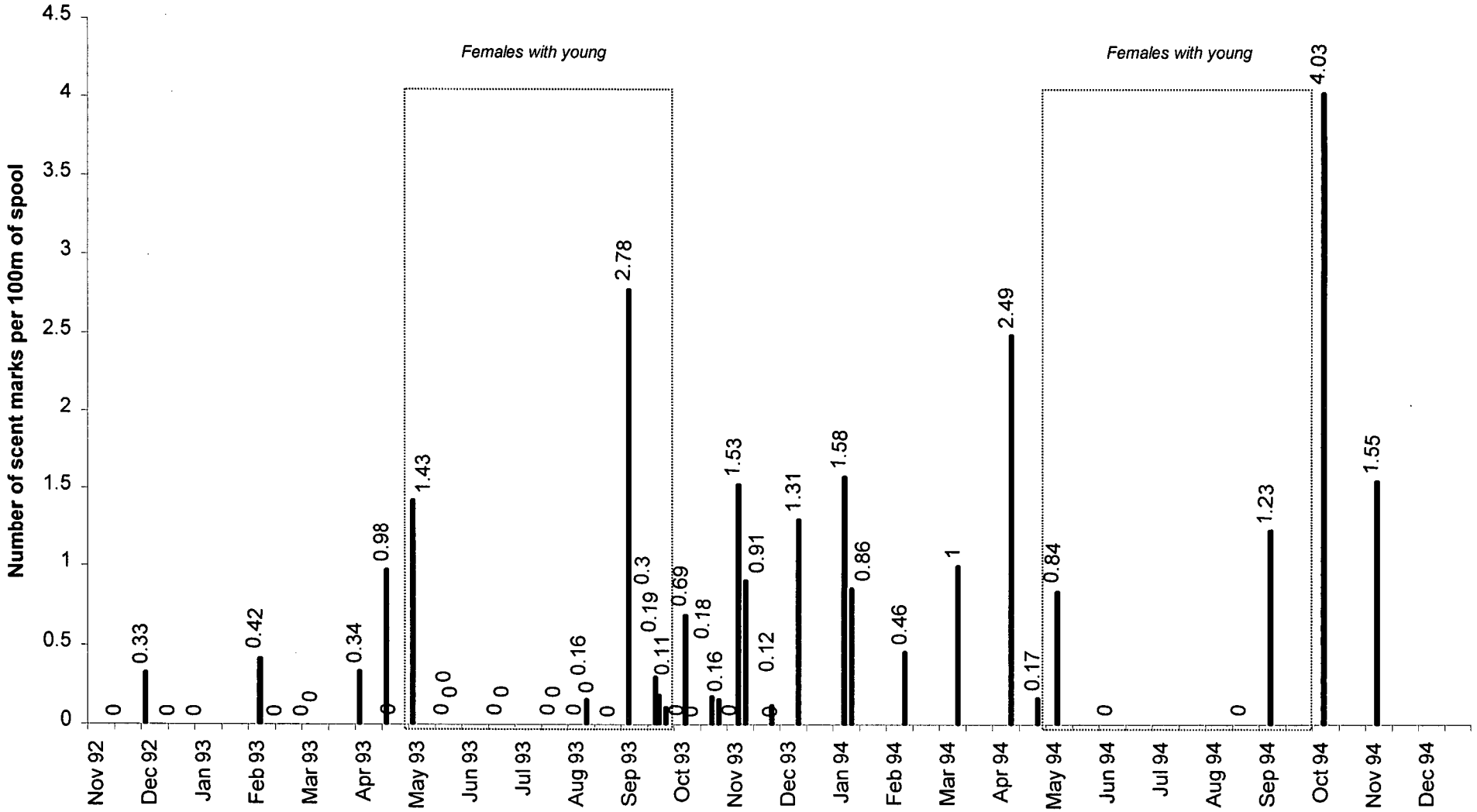
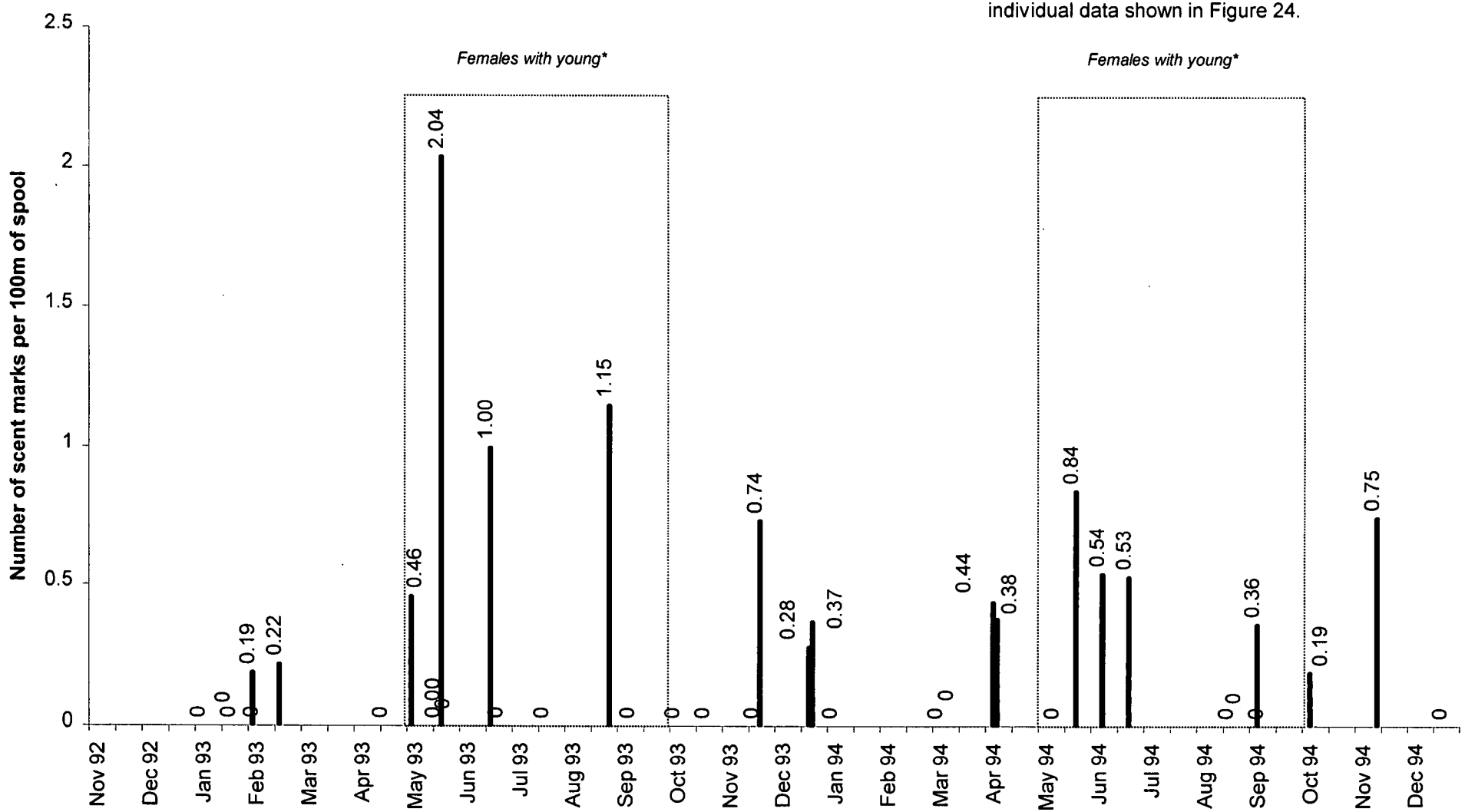


Figure 23. Scent marks per 100m of spool (Females)

* Note: not all females had young at this time -- individual data shown in Figure 24.



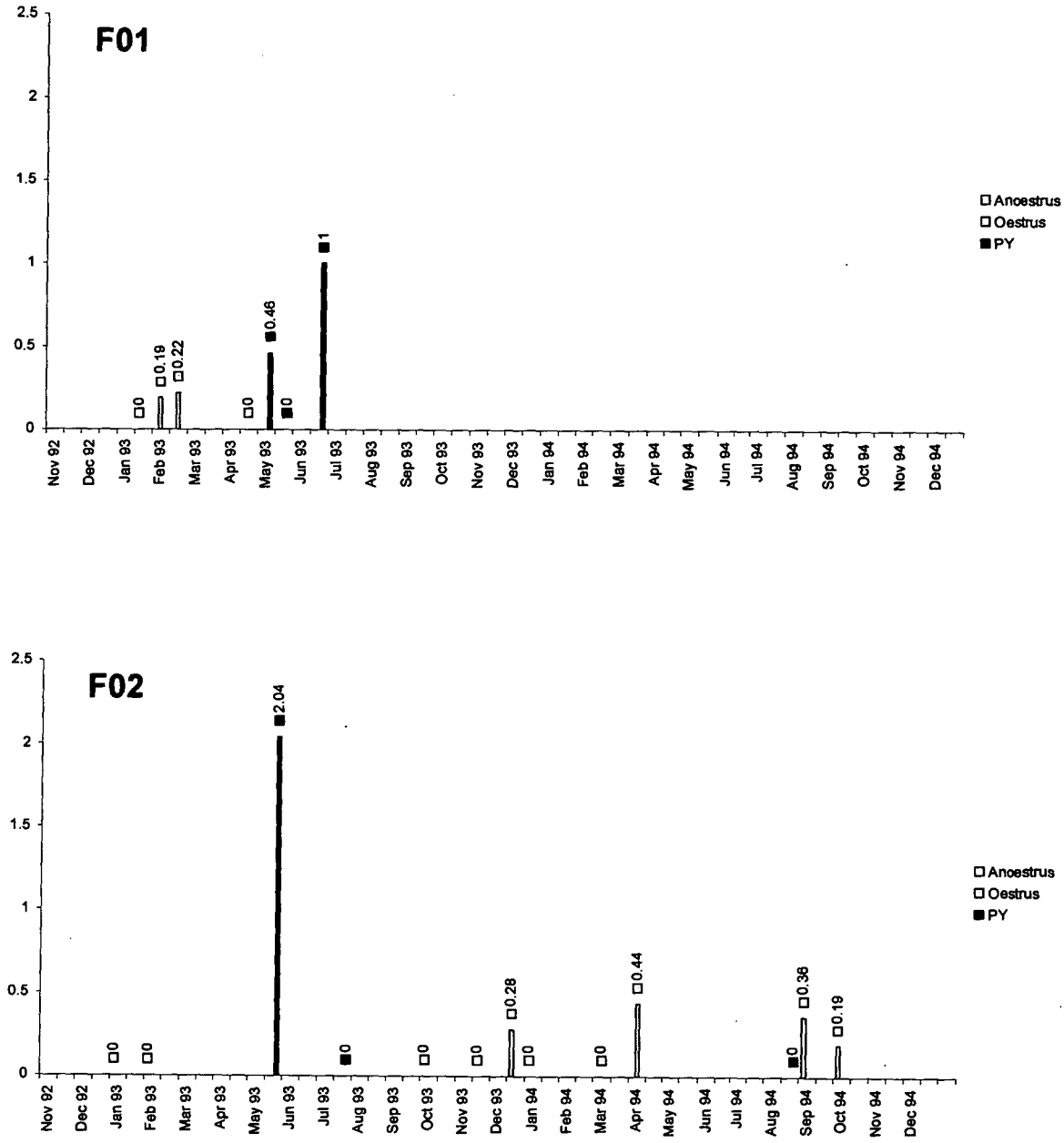
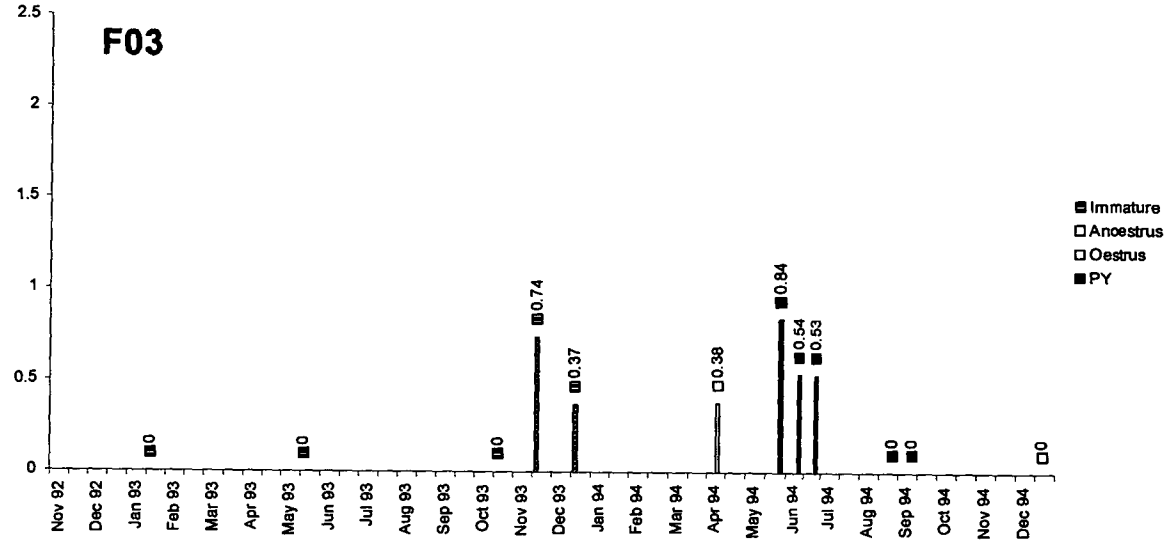


Figure 24. Number of scent marks per 100m of spool for each female in different reproductive states.



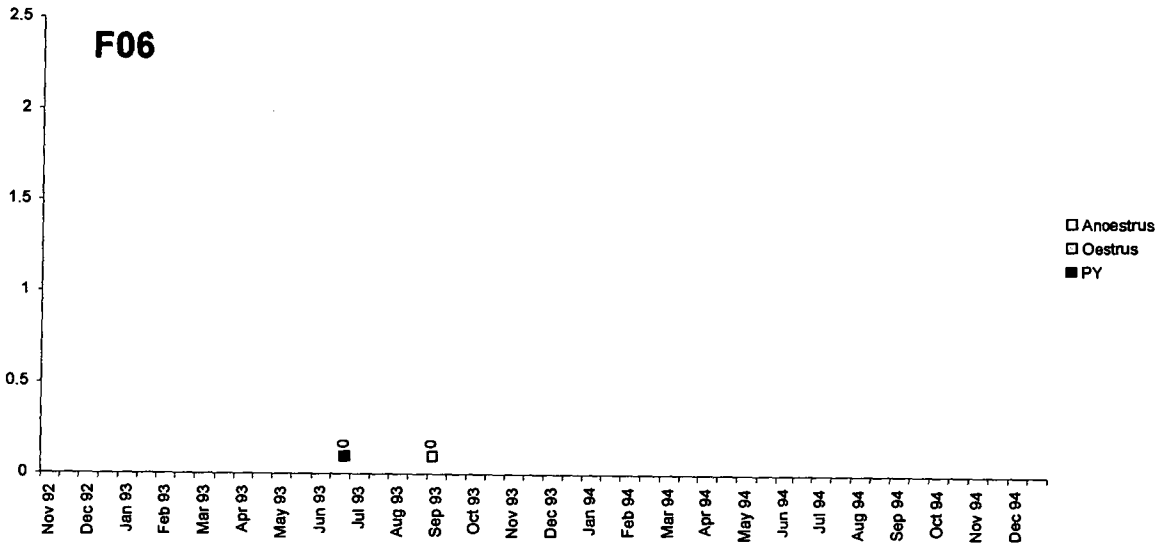
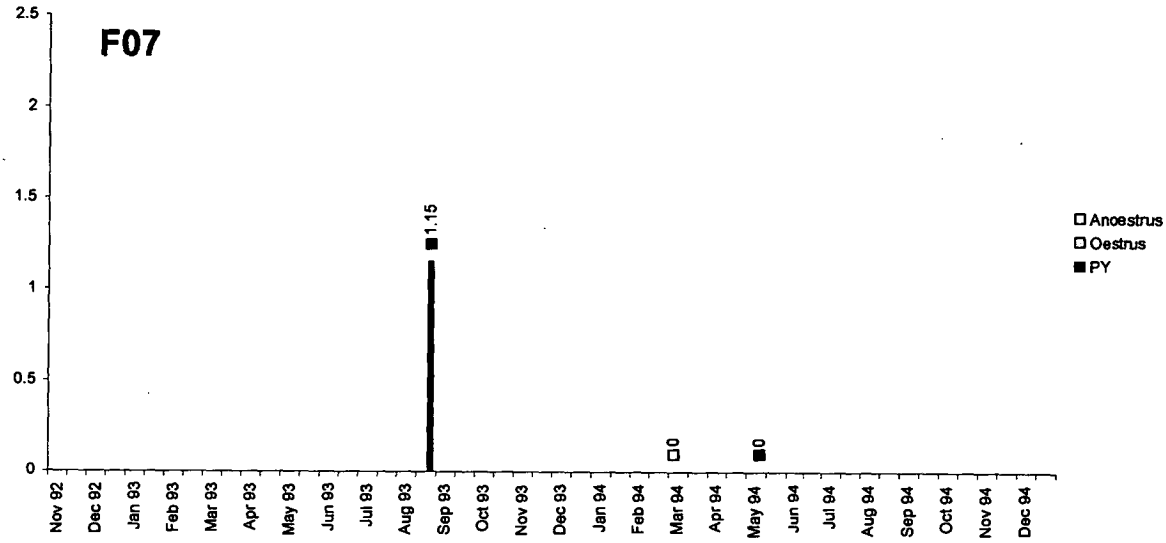


Figure 24. continued (females F03 and F06)



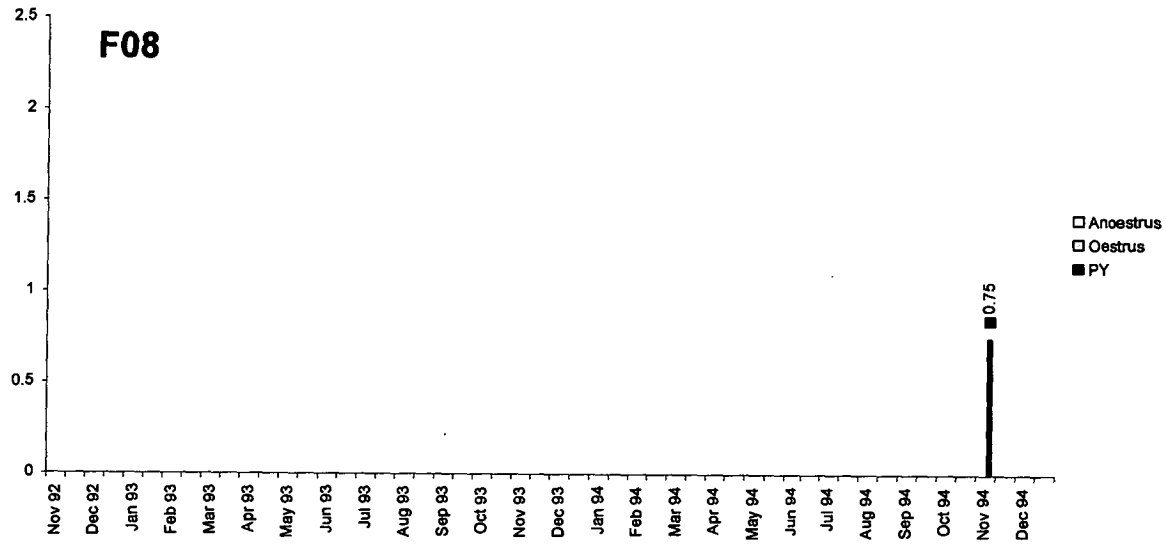


Figure 24. continued (females F07 and F08)

5.3.4. Location of sternal scent marks

Tables 41 and 42 show the distance of scent marks from the boundary of the home range of the marker for male and female possums respectively. Figures 25-30 show the home ranges of 3 male (M01, M07 & M09) and 3 female (F01, F02 & F03) possums and the position of scent marks within their ranges. For males the season in which each mark was made is shown, while the reproductive state of females is indicated for their marks. There is no evidence of any boundary marking by either males or females. In both genders scent marks appear to be scattered through the home range. Due to difficulties in accurately determining boundaries and because of the seasonal nature of marking statistical analysis of boundary verses hinterland marking was not conducted.

Table 41. Distance of scent marks from home range boundary: males*

	Season		No. of spools	No. of SM	Distance from edge of home range (m)				
					< 5m	5.0-9.9m	10.0-14.9m	15.0-19.9m	≥ 20m
M01	Dispersal	'92	3	1	1	-	-	-	-
	Pre-breeding	'93	5	0	-	-	-	-	-
	Breeding	'93	3	13	7	1	-	1	4
M07	Pre-breeding	'93	2	2	2	-	-	-	-
	Breeding	'93	2	0	-	-	-	-	-
	Post-breeding	'93	2	1	-	1	-	-	-
	Dispersal	'93	4	11	4	-	-	1	6
	Pre-breeding	'94	2	14	4	2	-	2	6
	Breeding	'94	3	13	2	-	-	-	11
	Post-breeding	'94	2	10	5	2	1	-	2
	Dispersal	'94	2	6	1	-	-	2	3
M09	Breeding	'93	2	0	-	-	-	-	-
	Post-breeding	'93	4	2	-	-	-	1	1
	Dispersal	'93	4	26	9	1	5	4	7
	Pre-breeding	'94	3	14	7	4	-	-	3
	Breeding	'94	2	3	-	1	1	1	-
Total				117	42	12	7	12	43

*Note: only animals with sufficient home ranges data, as listed in Chapter 4, are shown here.

Table 42. Distance of scent marks from home range boundary: females*

	Reproductive State	No. of spools	No. of SM	Distance from edge of home range (m)				
				< 5m	5.0-9.9m	10.0-14.9m	15.0-19.9m	≥ 20m
F01	Anoestrus	1	0	-	-	-	-	-
	Oestrus	3	2	2	-	-	-	-
	Pouch young	4	6	4	-	-	2	-
F02	Anoestrus	2	2	-	-	-	-	2
	Pouch young	2	1	-	-	-	-	1
	Anoestrus	4	1	-	1	-	-	-
	Oestrus	2	4	-	-	-	-	4
	Pouch young	2	0	-	-	-	-	-
	Anoestrus	1	2	1	-	-	1	-
	Oestrus	1	1	-	-	-	-	1
F03	Immature	3	0	-	-	-	-	-
	Immature‡	2	3	3	-	-	-	-
	Oestrus	1	1	1	-	-	-	-
	Pouch young	5	11	2	1	3	-	5
	Anoestrus	1	0	-	-	-	-	-
	Total		34	13	2	3	3	13

* Note: only animals with sufficient home ranges data, as listed in Chapter 4 are shown here.
‡F03 still immature, but pouch starting to form, nipples everted and skin above nipple has lost fur and become stained an orange-brown colour; body weight has also increased.

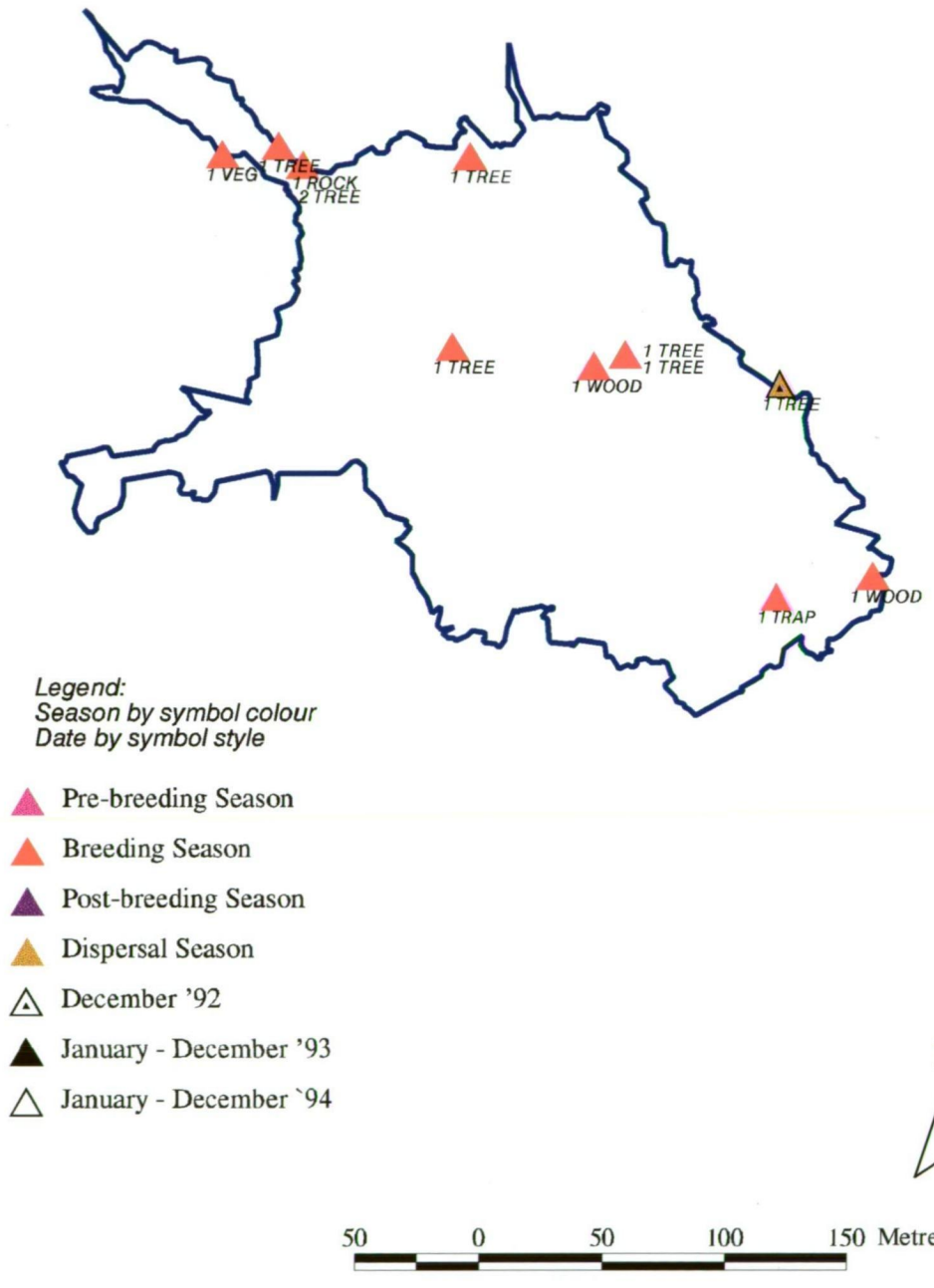


Figure 25. M01 home range showing scent marks by season.

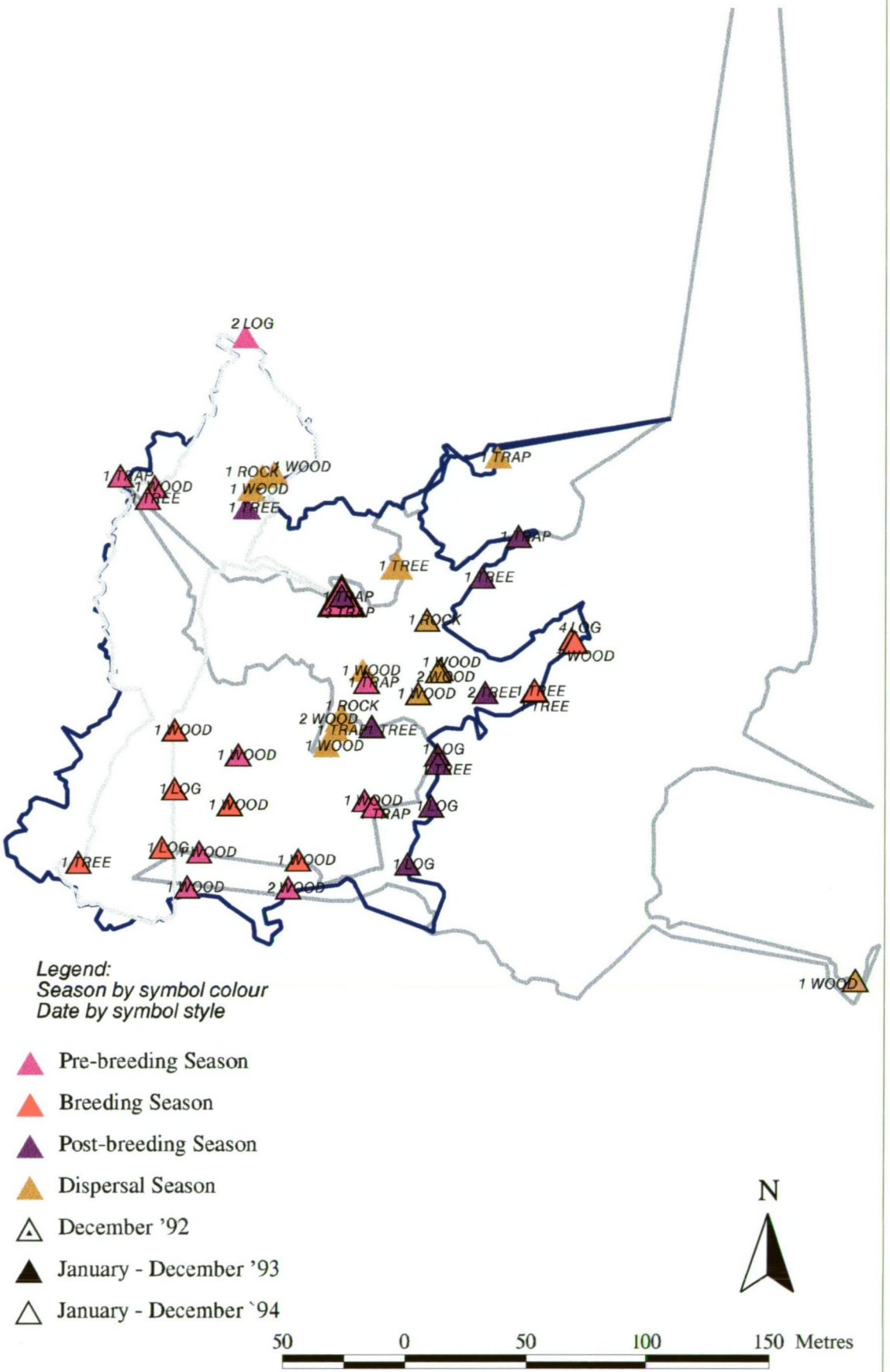


Figure 26. M07 home ranges showing scent marks by season.

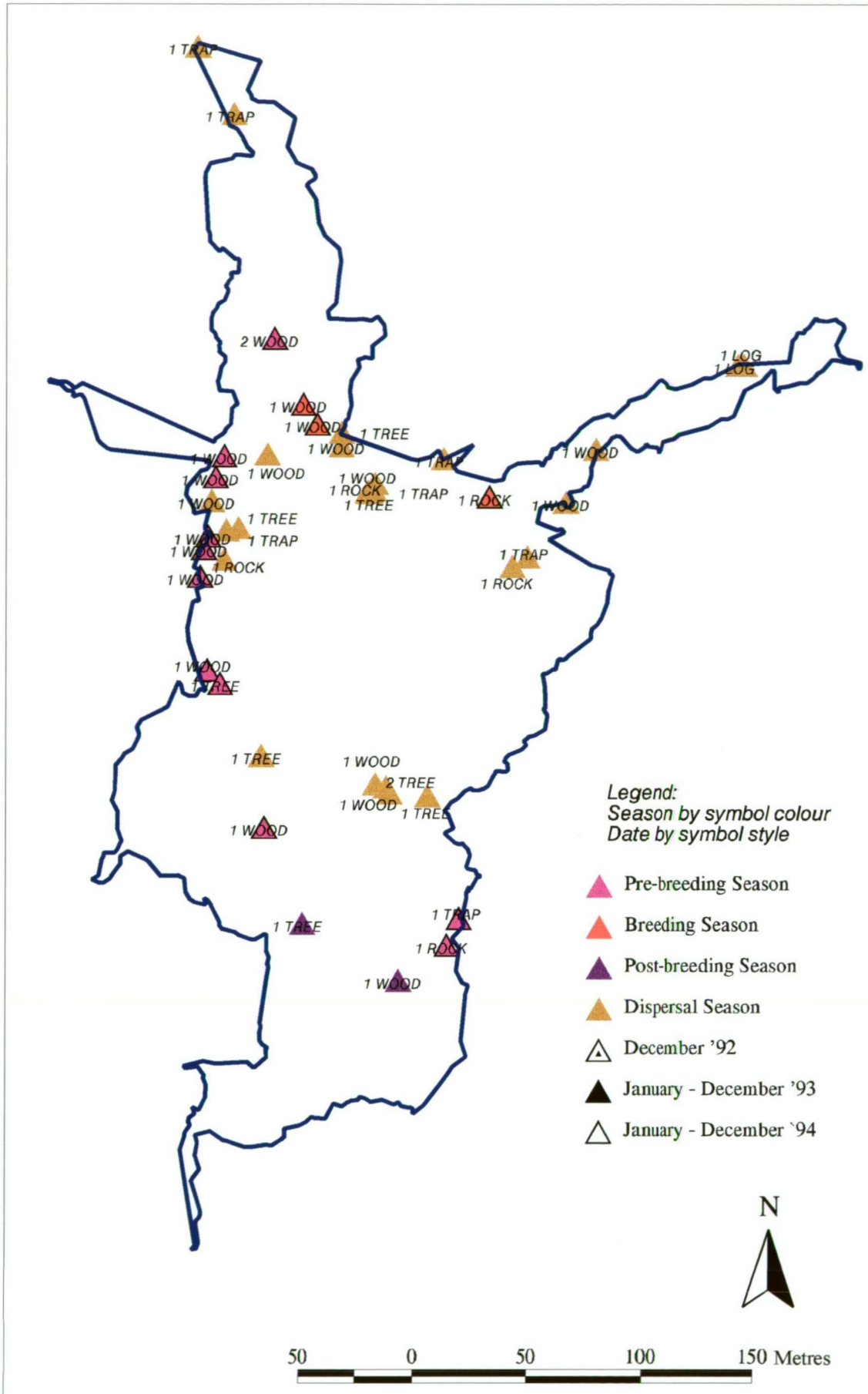


Figure 27. M09 home range showing scent marks by season.

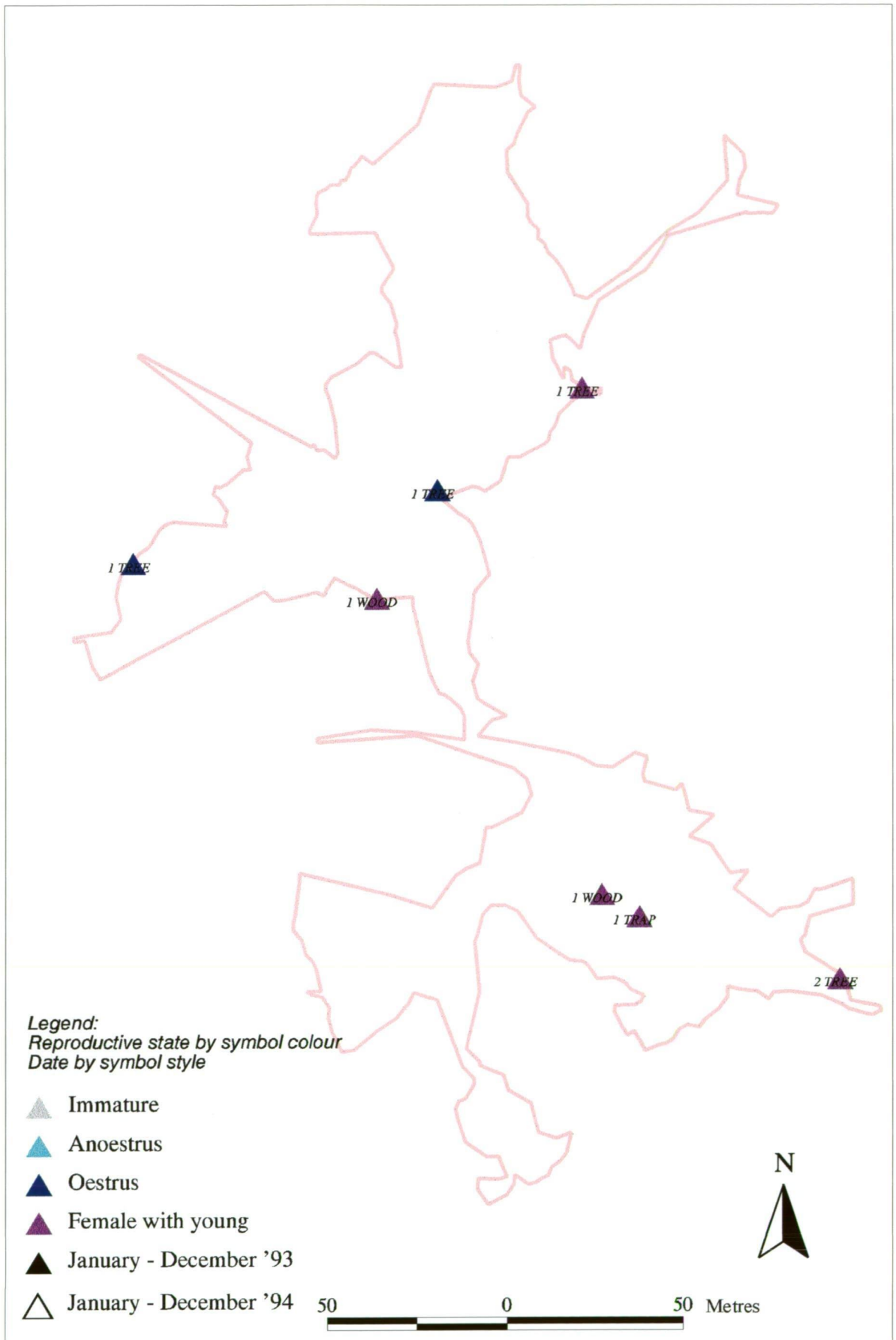


Figure 28. F01 home range showing scent marks by reproductive state.

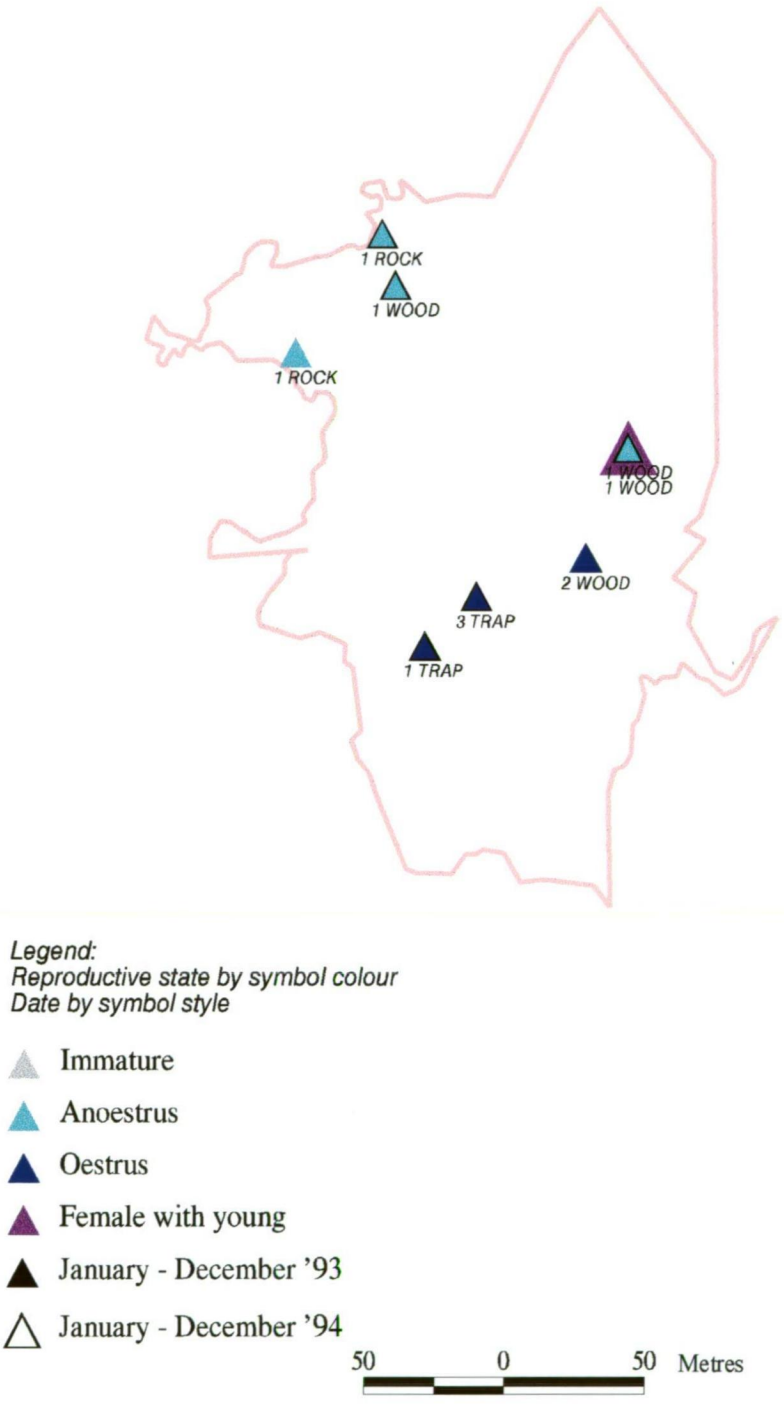


Figure 29. F02 home range showing scent markd by reproductive state.

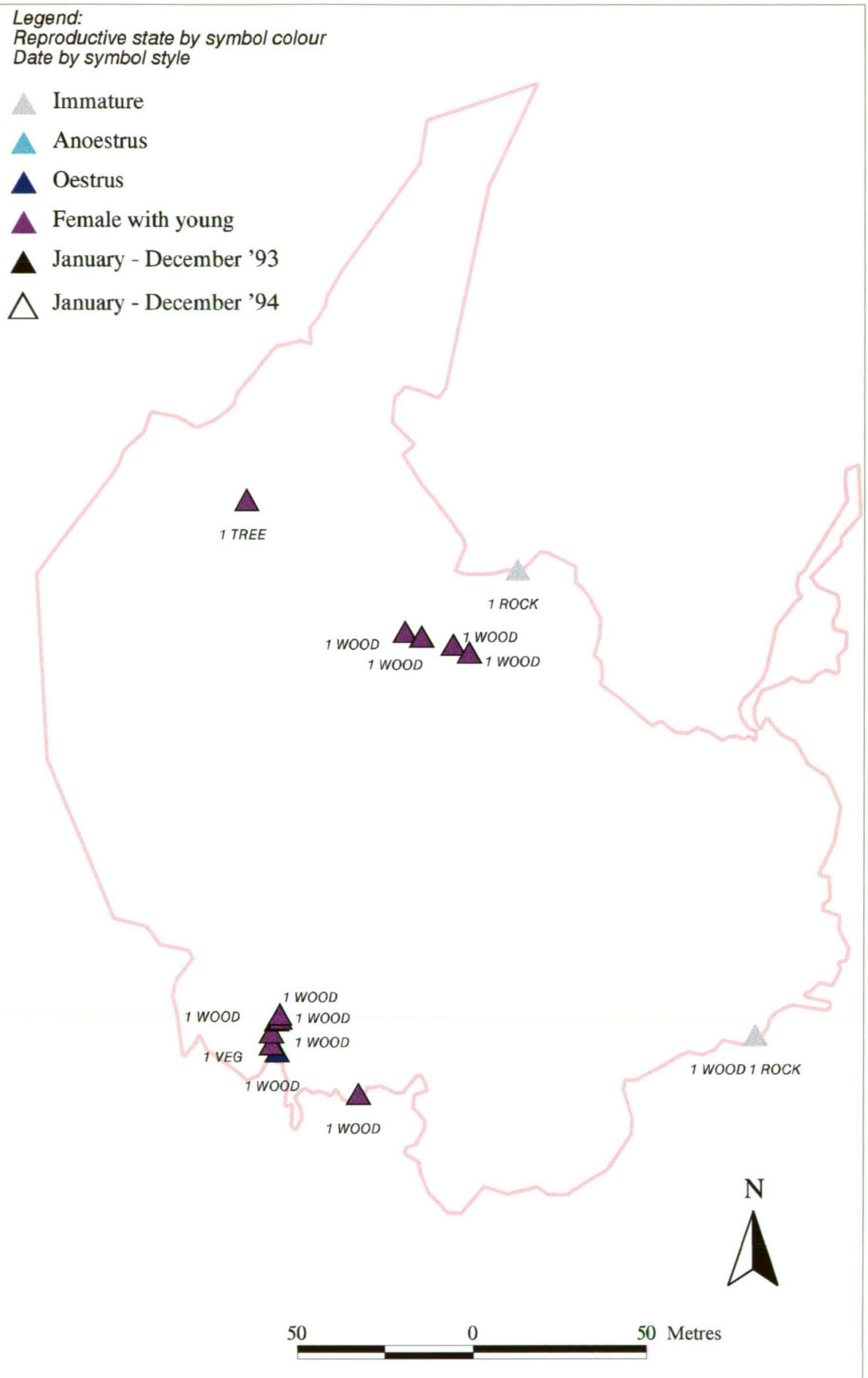


Figure 30. F03 home range showing scent marks by reproductive state.

5.3.5. Marking of den sites

Details of the behaviour of possums as they left diurnal den sites are given in Tables 43 and 44. Scent marking is highlighted.

Table 43. Observations of female possums in den trees.

	Date	Den	Observations
F02	11/10/94	499	• Head out of den entrance, looked around and pulled head back in • Repeated above for ~ 30sec • Emerged from den • Went up trunk, along a branch and into a neighbouring tree • Moved up branches of second tree and across into a third • Observation ended (not possible to observe animal) • Did not leave tree • Possum did not have pouch young or young on back • No marking observed
F03	10/10/94	494	• Emerged from den, followed by young who climbed onto back of mother • Moved in a spiral pattern up the truck and sat in branch opposite den entrance for ~ 30 sec • Continued moving up tree. • Observation ended • Did not leave tree • No marking observed
	13/10/94	284	• Emerged from den with young on back • Moved into branch above den entrance • Moved down trunk past den entrance and along braches to neighbouring tree • Observation ended • No marking observed
	8/11/94	508	• Emerged from den, young followed and climbed onto back • Sat sniffing and looking around • Young moved around, off and onto mothers back, climbed through braches eating leaves • F03 moved from entrance into branches with young on back • Observation ended • Did not leave tree • No marking observed
	11/11/94	284	• Emerged from den with young on back • Climbed up trunk and into a large branch, moved along to the end of the branch • Observation ended • Did not leave tree • No marking observed
	12/11/94	508	• Emerged from den, young followed • Sat on branch next to den entrance for ~10mins • Moved down trunk and sat on another branch before moving down the trunk to the ground • Observation ended (not possible to observe animal on the ground) • No marking observed
	21/12/94	493	• Emerged from den • Young not present • Sat outside den entrance and sniffed the air • Moved along branch and groomed with hind legs for ~ 5 mins • Continued along branch: **small sternal rub** • Moving around braches, occasionally stopping to groom: **possibly did a short chin wipe** (difficult to observe) • Moved back to trunk and climbed up tree • Observation ended • Did not leave tree

Table 44. Observation of male possum M07 at den trees.

	Date	Den	Observations
M07	14/9/94	488	• Emerged from den and returned immediately • 1 hr later emerged and immediately returned again • Observation ended ½ hr later.
			• Did not leave tree • No marking observed
	15/9/94	58	• Emerged from den • Groomed while sitting at den entrance (10 mins) • Returned to den. • Observation ended 1hr 40mins later (heavy rain started falling)
			• Did not leave tree • No marking observed •
	16/9/94	488	• Emerged from den and immediately returned • Emerged again 25 mins later: large sternal rub on branch opposite den opening • Moved up branch: **2 sternal rubs, 1 large chin wipe** • Moved further up branch: **4 large sternal-chin combinations** • Moved to end of branch; **1 sternal rub** • Possum spent much time trying to find a way of crossing into the neighbouring tree. • Rubbing and chinning appeared to be in frustration at not being able to cross from den tree to the neighbouring tree
	17/9/94	58	• Emerged from den and sat at entrance: **1 large sternal rub** • Moved out to a branch: **2 sternal rubs and 1 sternal-chin combination** on trunk • Moved onto trunk on the opposite side of the den entrance: urinated down trunk, followed by **4 sternal rubs** up the trunk • Returned to nest hole • Observation ended
	12/10/94	58	• Emerged from den, sat at entrance, occasionally lifting head to look around and/or to sniff • Started moving away from den entrance: **1 small sternal rub** on upper left side of den entrance • Moved into branch above nest entrance: **1 small sternal rub** on branch **and 2 short duration cloacal dabs** (ie held tail up and deliberately placed cloaca down on the branch) • Groomed (hind leg to scratch around hip area) • Turned around and sniffed area where cloacal dabs were made; made **another cloacal dab** • Moved down trunk to a lower branch • Continued down trunk to ground • Observation ended (not possible to observe animal on the ground)
	15/10/94	488	• Emerged from den, very alert (head up, ears pricked, occasionally looking around) • **2 large sternal rubs, 2 chin wipes**, and **another large sternal rub** on the left side on the den entrance • Moved down trunk to a large branch: **5 long sternal rubs** (possum appeared to be pressing down hard on the branch), sat up, then **2 more sternal rubs** in the same place • Moved along branch, sniffed and chinned a smaller branch • Moved back to branch near den entrance and then into braches near neighbouring tree • Observation ended ½ later, not observed to leave tree
	8/11/94	493	• Emerged from den, sniffed and looked around • Moved along branch: **1 small sternal rub** • Moved along branch and back again: **1 large sternal rub** • Moved back to original position on branch • Sat sniffing the air, sniffed branch: **2 chin wipes, 1 large sternal rub** • Immediately started moving down trunk and continued with out stopping until reaching the ground • Observation ended (not possible to observe animal on the ground)
	9/11/94	58	• Emerged from den and sat at entrance: **2 long sternal-chin combinations** on left side of den entrance, followed by **3 more sternal-chin combinations** • Moved onto branch above den entrance: sat and groomed • Turned around on branch and **wiped left and then right sides of chin** on branch • Moved down trunk to lower branches: **1 chin wipe** on a branch • Continued down through lower braches until reaching the ground • Observation ended (not possible to observe animal on the ground)

The results of this section need to be treated cautiously due to the limited number of animals observed (1 male and 2 females) and the short time period over which the observations were made (September to December). Despite the limitations of the data a number of points can be made.

Firstly, the male observed marked more often than either female. Upon emerging from his den he was observed to use a variety of scent marking methods including sternal rubbing, chinning, sternal-chin combinations and urination. The marks were made around the entrance to the den and on branches surrounding the entrance. M01 was observed to over-mark areas around the den entrances of trees 58 and 488 on different nights.

Detection of marking by females was made on one observation night only. No marking by either female was made during the months of September to November. During this time female F02 was observed at a den tree once. No scent marking was recorded during the den site observation, although one scent mark was located the previous evening using spool-and-line tracking. At this time F02 appeared to be in oestrus following the loss of her 3.5-4.5 month old pouch young 4-8 weeks earlier.

Between September and November F03 was carrying her young (born in May) on her back. F03 was not observed to engage in any form of marking during observations of den trees at this time. (It is not possible to know whether F03 marked any other part of her home range at this time, as there is no spool-and-line-tracking data. This is because it was not possible to use a spool-and-line device when a young was being carried). In December, at the end of the dispersal period, F03s' young was no longer with her and she was observed to mark around her den using sternal rubbing and chin wiping. Spool-and –line tracking the following night did not reveal any scent marking, however.

5.3.6. Sternal staining index

Figures 31 and 32 show the sternal staining indexes measured in mature male and female possums in the field over the duration of the field study. Figure 33 gives the average sternal staining index for male and female roadkill possums over four seasons. In Figure 34 the sternal staining index for field and roadkill females is shown with respect to their reproductive status.

In Figure 31 not all mature field males are shown. Only the Group A (ie M01, M04, M07, M08, M09 & M12) and Groups B (ie M03, M05, M11, M16 & M17) males from Table 30 in *Chapter 4* are shown. The Groups A males were considered to be the older, established resident males. The Group B males were the mature individuals that were caught only once during the study; these males were considered to be transient individuals. No data are shown for the mature Group B males of Table 30. These were the males considered to be younger, subordinate individuals whose home ranges overlapped with the older resident. These males were caught infrequently over varying periods of time during the study and because the data for these individuals were sporadic it was not possible to show any seasonal pattern in the sternal staining for these individuals.

A cycle of sternal staining is evident for the Group A males from the field site. In general the greatest level of development occurs in the pre-breeding period. Once mating has occurred there is a decline in the degree of sternal staining. The decline continues through the post-breeding period, but begins to increase again during the dispersal phase.

The same pattern is not evident in the mature roadkill males. There were no significant differences between the seasonal groups ($F_{(3, 100)} = 0.692$, $p=0.559$). It should be noted, however, that the roadkill males collected in this study were not necessarily comparable to the Group A males in the field study. The roadkill sample probably includes a variety of males, including older males with established home ranges, younger mature males without established home ranges and transient males searching for vacant areas to settle (see *Chapter 2. Histology of the Sternal Integument* for further details).

The female data classified by season should be treated with some caution as not all females examined at a particular time of the year were in the same reproductive state. Among the females in the field study there is some variation in the general pattern of sternal staining in the different years. In 1993 the greatest levels of sternal staining occurred during the breeding season. A similar pattern occurred in 1994, although two females, F02 and F03, had greater sternal staining in the pre-breeding period. There is evidence that females F02 and F03 came into oestrus earlier than most* of the females with sternal staining that peaked in the breeding season (*Note: the timing of oestrus was not known for all females). In general sternal staining decreases after the breeding season, showing its lowest levels during the post-breeding period. Sternal staining begins to increase during the dispersal phase and continues to increase in the pre-breeding period. Among the mature roadkill females the pattern of sternal staining is somewhat different. The greatest amount of staining is seen in the pre-breeding period, after which there is a decrease in the breeding season. During the post-breeding period the level of staining increases again, but this is followed by a decrease in the dispersal phase. There were no significant differences in the amount of sternal staining between the seasons ($F_{(3, 31)} = 2.732$, $p=0.063$), although differences in the pre-breeding and dispersal periods approached significance (Tukey test $p=0.060$), with the sternal staining of roadkill females during the pre-breeding season being greater than during the dispersal period. It should be noted that in each of the seasons there is a mixture of mature females in different reproductive states (ie anoestrus, oestrus and with young). If the amount of sternal staining is related to the reproductive state of females displaying the roadkill females by season may mask any differences between females of different reproductive states. Examination of females in the field study by reproductive state reveals that immature females had significantly less staining than all categories of

mature females ($F_{(3, 87)} = 11.394$, $p << 0.001$. Tukey Test: immature < anoestrus & oestrus, $p < 0.0001$, immature < females with young, $p = 0.001$). Among the mature females in the field study there differences in the amount of sternal staining evident in the histograms are not significant ($F_{(2,65)} = 2.674$, $p=0.077$). Females in oestrus, however, had the greatest amounts of sternal staining and this was close to being significantly greater than in females with young (Tukey Test: oestrus > females with young, $p=0.062$). In females with young and females in anoestrus the level of sternal staining was lower. There was no significant difference between anoestrus female and females with young or between anoestrus and oestrus females (Tukey Test: anoestrus & female with young, $p = 0.257$; anoestrus & oestrus, $p = 0.628$). This data corresponds well with the seasonal data from the field. That is, during the pre-breeding and breeding periods when most females were in oestrus the level of sternal staining was at its highest. In the post-breeding and dispersal seasons when females have young or are in an anoestrus condition the level of sternal staining is lower.

Among the mature roadkill females reproductive state does not reveal any significant differences in the amount of sternal staining ($F_{(2,28)} = 0.187$, $p = 0.831$). Again this results should be treated cautiously as the sample size is small and there were some difficulties in classifying the reproductive state of some of the roadkill females (see *Chapter 2. Histology of the Sternal Integument)*.

Figure 31. Sternal Staining Index - Mature Males (Field Data)

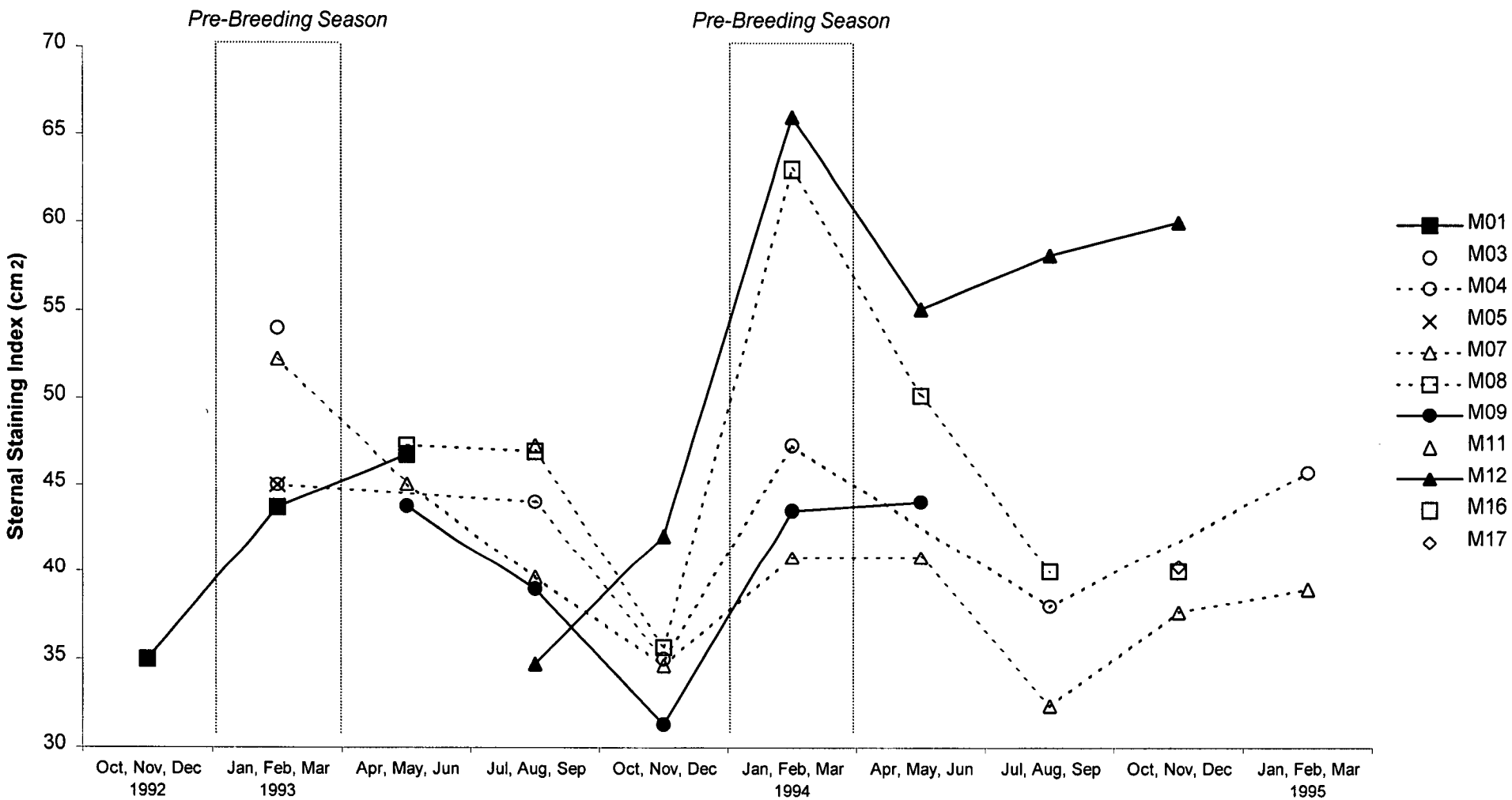
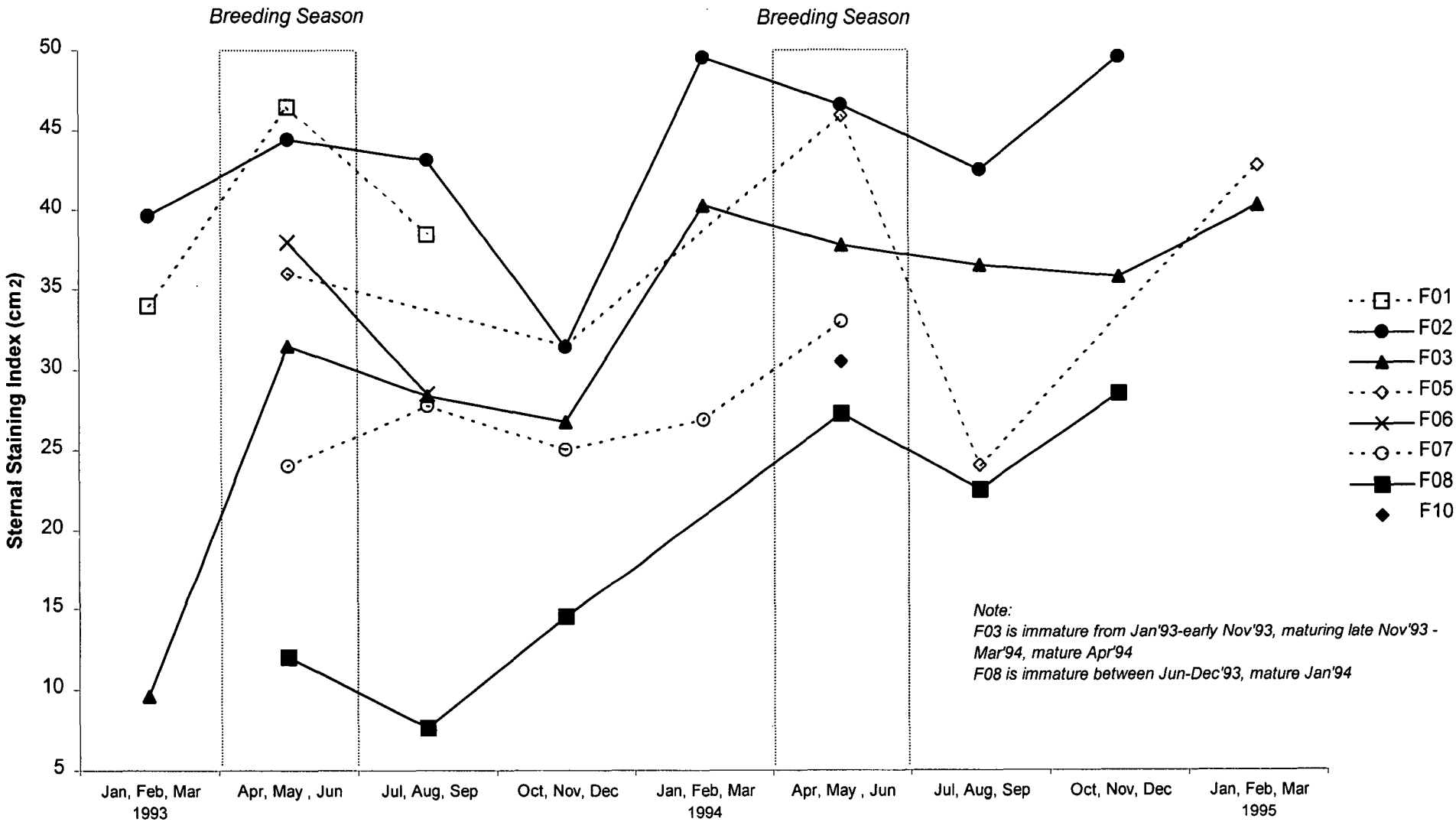
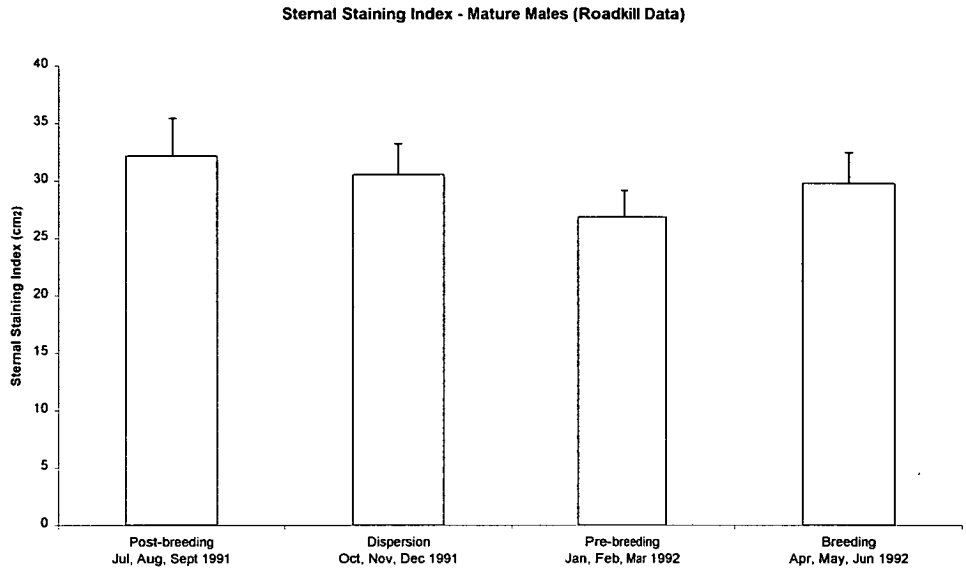


Figure 32. Sternal Staining Index - Mature Females (Field Data)





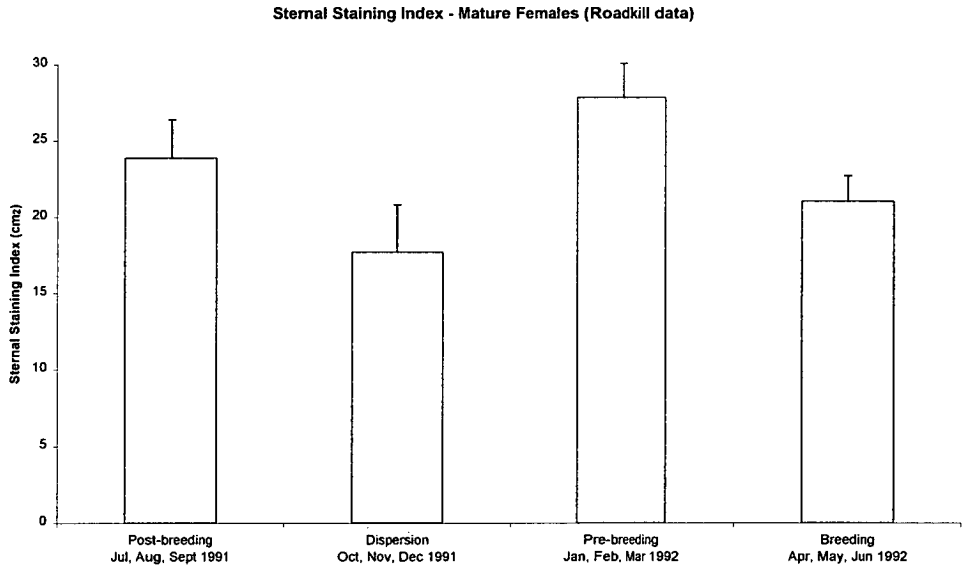
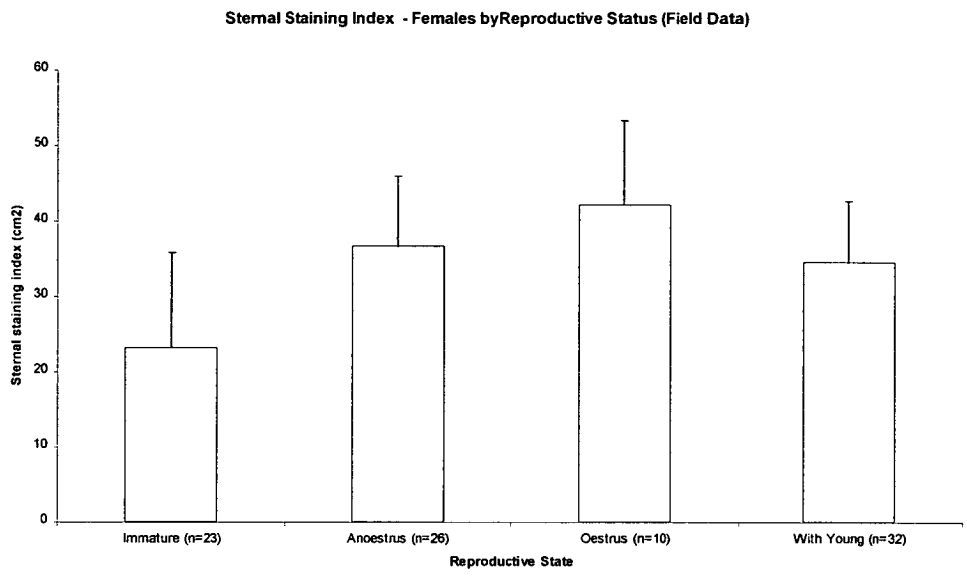


Figure 33. Sternal staining index (cm^2) for mature male (n = 104) and female (n = 31) roadkill possums over four seasons.



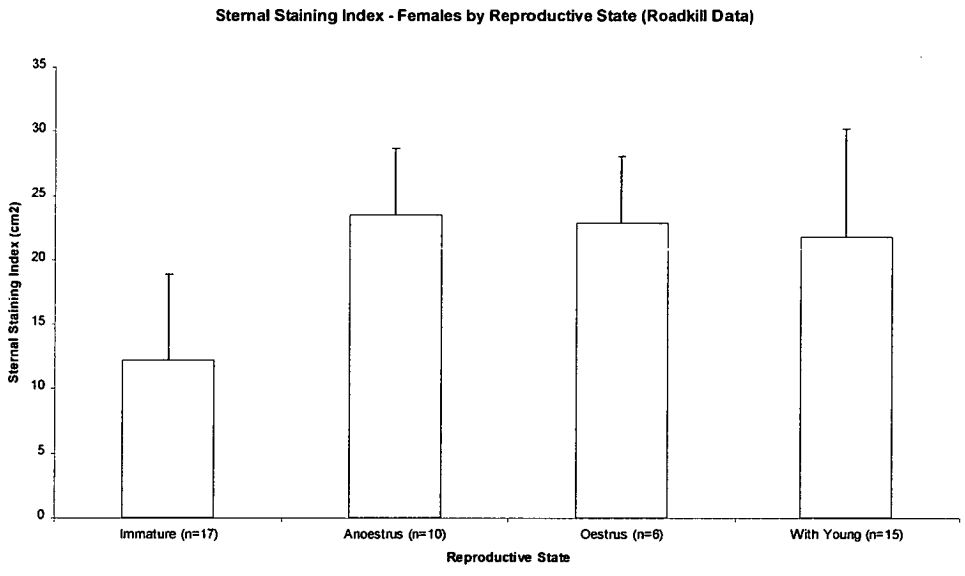


Figure 34. Sternal staining index (cm^2) for field and roadkill females by reproductive state.

5.4. Discussion

A number of new observations on the sternal gland and scent marking behaviour in the brushtail possum have been made. These will be discussed together with results supporting earlier studies of scent marking in this species. Discussion of the results is divided into two sections. The first section will consider the location of scent marks in the home ranges of possums at the site. The second will address maturity, gender and seasonal differences in sternal gland scent marking. Each section will be discussed with respect to what is known about the biology and ecology of the possum. Some discussion of the possible functions of sternal gland scent marking will occur here, although most of the discussion on function of scent marking in the brushtail possum can be found in the following section, Chapter 6.

5.4.1. Location of sternal scent marks

Brushtail possums were recorded marking on a variety of objects in the environment. These included tree trunks, branches of shrubs, clumps of grasses, fallen logs, fallen sticks, branches and bark on the ground, pieces of wood (from past logging activities), rocks, and on objects (ie. rock, bark etc.) used to cover traps. Winter (1977) reported a similar range of objects being marked using the sternal gland. Most of the marks were made on objects on the ground or close to the ground. This is not unexpected given that the possums are only semi-arboreal and spend much of their time on the ground (Guiler 1957).

The majority of scent marks detected on trees were at a height of less than 75cm from the ground. Spool-and-line tracking revealed that the possums scent marked trees while standing on the ground or as they began to climb up. Very few scent marks were made higher on the trunks of trees. Although detection of scent marks among the branches of trees was not possible using the methods employed in this study previous studies do not report a high level of sternal marking in trees. Winter (1977) observed that most scent marking involving the sternal gland in male possums were associated with the bases of trees as an individual walked past the tree. Marking higher up trees was more often done using the chinning behaviour. Some sternal rubbing around den entrances and on surrounding branches was observed during spotlighting of radio-tagged individuals in the current study. Winter (1977) also observed that sternal marks were deposited on den trees by possums leaving and returning to dens.

A range of trees of various age and species were marked. All of the *Eucalyptus* species that were common at the site were marked. There is not much evidence that the trees marked in this study were marked because of their value as a resource, either as a den tree or a feeding tree. Very few of the marked trees in this study were known to be used as den trees or feeding trees by either the marker or another individual. Only 8% of trees marked using the sternal gland were identified as den trees that belonged to the marker or another individual. A third of the marked trees were observed to have been climbed, and possibly used for feeding, by the marker at the time of marking or on another night, or by another possum at some stage. Winter (1977) reported that important resource trees (ie den trees and feeding trees) were common targets for sternal marking by both males and females and that although scent marking occurred throughout the home range den trees used by the resident and other males where focal points for marking. Marking of nest sites, particularly when they are in limited supply, has been recorded in other mammals (eg golden spiny mice, *Acomys russatus* (Rozenfeld *et al* 1994)). In the current study the lack of association between sternal scent marks on or near trees that were used as den or feeding trees may be due to the low number of observations of individuals at a particular tree. During the study 517 trees were recorded as having an association with a possum, ie the possum went past

the tree (within 0.5m), up the tree and/or scent marked the tree. In only 29% (ie 150/517) of cases was a tree recorded as being "used" more than once. More frequent spool-and-line tracking may have shown that more of the marked trees were used as den site or feeding trees by the marker or another possum.

Another reason for the apparent low level of scent marking on den trees in this study may be directly related to the technique used to detect scent marking. Winter (1977) observed (in males) that a temporal pattern of sternal scent marking. Males often showed a peak in marking early in the evening that was concentrated on and around the den tree. A second peak was observed before dawn as males returned to a den. Again this marking was concentrated on the den tree. In the current study possums began their nightly activities in a hessian sack next to the trap they were captured in the previous night. It was observed on a number of occasions that both males and females would engage in a high level of marking in the first part of the spool-line. This is clearly visible in Figure 30 as clusters of scent marks in two areas. On both these occasions females F03 made all these marks in the first 30 metres of spool line. It is probable that marking in the first part of the spool-line is analogous to the marking of den trees early in the evening in Winter's study. Furthermore, it was mentioned in *Chapter4* that many of the possums in this study covered distances greater than 1 kilometre during the night and that spool-lines often ran out before the animal returned to a den site. In the few observations were a possum did return to a den, however, scent marking was not observed. It is likely that scent marking of den sites was much more common than the results report due to the methods used in the study.

Marking by possums as they leave their den may serve two functions. Marking may identify the den site as being occupied or owned thus deterring other individuals from entering while the resident is absent. Marking early in the evening before starting nightly activities may serve to "boost the confidence" of the marker as they set off. Saturation of the area with a familiar odour may enhance increase the marker's confidence and enhance their ability to deter intruders they may encounter. Mykytowycz 1972 (cited in Mykytowycz 1972) states that "only within an area where its own odour prevails will an animal behave freely and participate in breeding activities" and observations and experiments have shown resident individuals are consistently more successful than intruders during agonistic encounters (Eisenberg & Kleiman 1972).

A possible reason for a low level of marking on trees used for feeding may related to the observation that more than half the food obtained by possums in undisturbed and regeneration wet sclerophyll forests in Tasmania came from plants growing on the ground (Statham 1984). This is in contrast to the observation by Freeland and Winter (1975) in an open grassy forest in Queensland that possum spend 66% of their feeding time eating mature eucalypt leaves. These differences may account for the large percentage of scent marks located on the ground in this study.

Although there was not much evidence of marking at known den or food trees the observation that both males and females marked on or near traps as they moved around their home ranges supports the theory that marking has a resource protection role. To increase trapping success during the study traps were baited and wired open during field trips to allow possums to enter and feed without being trapped. Scent marks on and around traps may have been placed to deter intruders from a regular food source.

In this study there was no evidence of boundary marking by possums of either sex. Winter (1977) did not observe marking of the home range boundary by males in his study. It can be assumed that females did not perform boundary marking either, although Winter did not specifically report this. In this study and Winter's scent marks were scattered throughout the home range of individual possums rather than predominantly on home range boundaries. There is some evidence that important resources (ie dens and food) are focal points for marking. The lack of boundary marking supports the observations by Winter (1977) that possums do not have territories which they actively defend from intrusion by

other individuals and that a large number of agonistic interactions occur at focal points such as den and food trees and in the vicinity of oestrus females.

Male and female possums were both observed to over-mark in this study, although it did not occur often (10/169 marks = 6%). The scarcity of over-marks may be due to a couple of things working separately or in combination. Firstly, the tracking pattern may not have been frequent enough to pick up the extent of over-marking. It is not possible to know how long the "message" in the scent mark remains. Marks may be relatively long lasting and over-marking to "refresh" the mark may not be required very often. The duration of scent marks and therefore the rate of over-marking may be effected by the weather, with rain causing scent marks to be washed away, and/or hot weather causing volatile substances to evaporate more readily. Given that over-marking by the same individual was recorded to have occurred twice within a 3 day period (M09 12/4/94 and 15/4/94) one might be drawn to conclude that marks do not persist for an extended period of time in the environment. Another possible explanation of this instance may be that another individual marked the same spot between the dates when it was marked by M09. M09 may have marked again so soon because his first mark was no longer present.

Over-marking can be grouped into two major categories: re-marking and counter-marking. In the few instances of over-marking recorded in this study both types of marking were recorded, ie individuals marking over their own marks, and individuals marking over marks made by other possums. It should be noted that, although the data collected in this study shows that both re-marking and counter-marking occurred, it is possible that other possums marked at sites classified as re-marks. The methods used in this study were not able to detect all marks made by all individuals at the site. Despite possible classification errors it is evident that possums do engage in re-marking. Observations of male M07 leaving the same den site on different evenings revealed that re-marking of specific places on the den tree did occur. The function of re-marking is most likely to maintain an odour presence at a particular site in the home range, particularly an important resource such as a den. Because odours evaporate and their chemical composition changes over time (Regnier & Goodwin 1977) re-marking the same area will renew and maintain the odorous message contained in the original mark.

Counter-marking did not occur often in Winter's (1977) study. Males were observed to scent mark over marks made earlier in the night by another male, but females were not observed counter-mark. The most common observation of a male counter-marking occurred in situations where a male climbed a tree containing an oestrus female after marking the base. Counter-marking occurred when a second male arrived and also marked the base of the tree. In the majority of cases the second male did not climb tree indicating that the mark of the first male had a deterring effect. Without knowing the context of over-marking observed in the current study it is difficult to discuss the observations of over-marking further. Many of the second marks were made months after the initial mark and it is doubtful that any olfactory message from the first mark was still present at the time of the over-marking. If scent marks function by informing an individual that an area is occupied it is unlikely that the information contained in the mark last for extended periods of time. Evidence for this is provided by the observation that areas left vacant following the disappearance of the resident are soon taken over by other possums. The observation of three individuals marking the same area over an extended period of time, however, suggests that over-marking occurs at important sites within the overlapping home range of animals. It is unlikely that the location of the scent marks in the same area was due to chance.

Over-marking may not be the best description of what was observed in the brushtail possum. In all the observations the second mark was usually placed immediately adjacent to the first mark. There was very little evidence of overlap and no marks were recorded to completely cover the original mark. This has implications for the message that may be conveyed by the scent marks. Male golden hamsters (*Mesocricetus auratus),* for example, have been shown to be able to remember both scents in situations where marks do not

overlap, but to only remember the top scent and not the bottom when scents overlap partially (Johnston *et al* 1994; Johnston *et al* 1995; Wilcox & Johnston 1995). Johnston *et al* (1994) have proposed that counter-marking can have three results. Firstly scent blending may occur, where the odour qualities of the two marks may blend to create a new scent. This scenario would result in the production of a group scent. Examples of this type of counter-marking are seen in a range of mammals that live in social groups, such as rabbits *(Oryctolagus cuniculus)* (Mykytowycz 1968) and meerkats *(Suricata suricatta)* (Moran & Sorensen 1986). It is unlikely that counter-marking in the solitary brushtail possum functions to produce a group odour. The second result of counter-marking may be that the scent of the individuals may remain distinct. The outcome of this is the creation of a chemical bulletin board where individuals may come to find out about others or advertise themselves (Johnston *et al* 1994). If this is the purpose of counter-marking in the brushtail possum it may have been expected that counter-marking would have been detected more often and that all of the individuals at the site would have engaged in such marking. The third outcome of over-marking is that scent masking may occur, that is, the scent of the second marker prevents access to other individuals to the odour of the first mark. A masking hypothesis has been proposed for animals that scent mark to advertise their dominance status and/or use scent for territorial or home range defence (see Johnston *et al* 1994; Hurst 1987, 1990). This third suggestion is the most likely explanation of counter-marking in the brushtail possum. Possums have been shown to have intrasexual dominance hierarchies and have home ranges that are exclusive to same-sex individuals of similar status (Winter 1977).

5.4.2. Maturtity, gender and seasonal differences in marking

Maturity, gender and seasonal differences were found in sternal gland marking and factors associated with marking in the brushtail possum. Many of the results the show close correlations with data collected in this and previous studies on the biology and ecology of the species.

A number of observations highlight the relationship between maturity, the sternal integument and sternal gland scent marking. In the limited number of observations of immature individuals, using spool-and-line tracking, sternal gland scent marking occurred infrequently. No scent marking was observed in the male possum that had not reached sexual maturity. The immature female that was tracked started scent marking only during the months when she was observed to be approaching sexual maturity. As her pouch began to develop and her body weight increased the fur across her sternum became increasingly stained with secretion and she started to scent mark her home range. Before this time she was not observed to mark her home range. Winter (1977) did not observe scent marking behaviour in female possums before that age of 8 ½ months. Sexual maturity in females is usually achieved during their second year (Tyndale-Biscoe 1955; Pilton & Sharman 1962; Gilmore 1969), although oestrus cycling frequently begins at 12 months of age (Kean *et al* 1964; Smith *et al* 1969) and has been recorded in females as young as nine months (Pilton & Sharman 1962). Observations of young male possums scent marking have not been recorded until 21 months of age (Winter 1977), although this report may be due to lack of observations of young males. The development of scent glands and marking behaviour is often associated with approaching sexual maturity (Johnson 1973; Brown 1979; Stoddart 1980b). Similar observations have been made in many mammalian species including the rabbit *(Oryctolagus cuniculus)* (Mykytowycz 1965a, 1966a & b) and gray-tailed vole *(Microtus canicaudus)* (Wolff *et al*1994).

Among mature individuals a number of differences between the sexes were observed. Males were observed to mark more often using the sternal gland than females, and the rate of marking (ie number of marks over a given distance) was higher among males than females. Over half the sternal scent marks made by males were deposited on or within two

metres of a tree or a trap. Only a third of female scent marks were made on or close to trees or traps — the majority were made on objects on the ground. The size of scent marks on trees did not differ between the sexes. Scent marks on all other objects, however, were generally larger in males than females. This corresponds with the observation that sternal gland marking by females was never as vigorous as marking by males (Winter 1977). In females sternal marking usually consisted of a single quick light rub on the substrate and it was often difficult to know whether marking has taken place, whereas in males it was an obvious, firm and deliberate action that was often repeated (Winter 1977).

A number of seasonal differences in the timing of marking between and within each gender were observed. As occurs over most of its range (see Bolliger 1940; Tyndale-Biscoe 1955; Dunnet 1956, 1964; Gilmore 1969; Crawley 1973) possums at this site had a main breeding season in autumn during which the majority of females come into oestrus and mated. Sternal scent marking in males did not occur frequently once females at the site had mated or during the period when young were in the pouch. Most scent marking in males occurred in the dispersal, pre-breeding and early part of the breeding season. This pattern can also be seen in the sternal staining index. Sternal staining was highest for males in the pre-breeding period.

For females most marking occurred once they had a young in the pouch. A lower level of marking occurred during the dispersal phase and during the pre-breeding and breeding seasons in oestrus females. The observations of the sternal staining in females in this study do not correspond with the pattern of marking in females in the way that it did in males. On the contrary the sternal staining in females shows *less* development during the period of greatest sternal marking, that is, during the time when they were carrying young. Sternal staining is greater during the period of oestrus at the beginning of the breeding season, although some scent marking was observed in females as this time it was relatively low compared to when they were carrying young.

The observations of sternal scent marking in female possums in this study are somewhat different to the observations made in Winter's (1977) study. Sternal scent marking by females was reported by Winter to occur predominantly between the first and last time a young was seen riding on its mother's back. Young start riding on their mothers back when they are approximately 4-5 months old (Dunnet 1956, 1964; How 1972) and continue to do so for 1-2 months before becoming independent. It should be noted that in the current study use of spool-and-line tracking prohibited observation of females carrying young on their back. Observations of a female leaving her den site while carrying a young on her back, however, did not reveal any scent marking in females at this stage of the reproductive cycle. Unlike the current study Winter did not observe a high level of sternal marking in females carrying pouch young. Possible explanations for this discrepancy between the two studies is probably related to differences in the resources contained within and required by the individual possums at each study site. This hypothesis will be discussed further in Chapter 6 when the possible functions of scent marking in the brushtail possum are discussed. It should be noted, however, that there are a number of differences between this study site and Winter's. The autumn breeding season at Winter's site started earlier than in this study and the animals in Queensland were more fecund. Furthermore there was a distinct secondary spring breeding season at Winter's site which was not observed at Mt Morrison. The results of these differences is that unlike the four distinct phases of breeding in the current study, the reproductive stages and period of dispersal in Winter's study are repeated and overlap to some degree. These factors need to be considered when comparing the two studies.

Another interesting observation, which probably provides some explanation for the discrepancy described above, relates to the reproductive success of females in this study. As mentioned in *§4.3 Results* of *Chapter 4*, in two autumn breeding periods only two of seven young born to five females survived past the pouch stage. Previous studies have reported mortality of young in the pouch is low (Dunnet 1964; How 1972). Among the range of differences between the successful mothers (females F01 in 1993 and F03 in 1994) and the unsuccessful mothers (females F02, F06 & F07) it was noted that the successful

females had higher rates of sternal gland marking when carrying their pouch young than the unsuccessful females (see Figure 24).

The results of this part of the study support earlier studies of scent marking in the brushtail possum. A number of new observations on the sternal gland and scent marking behaviour in this species have also been reported, specifically in relation to the timing of scent marking. The function of scent marking in the brushtail possum will be discussed in the following section, Chapter 6.

Chapter 6. Discussion

This study has increased knowledge about gender and seasonal differences in the sternal integument and sternal gland scent marking in a natural population of brushtail possums. In *Chapter 1* the importance of integrating knowledge about the ecology and behaviour of a species with information about scent organs and scent marking behaviour in order to understand the possible functions of odours was emphasised. The purpose of this final chapter is to synthesise the large amount of ecological and behavioural information available for the brushtail possum with data collected in this study with the aim of better understanding the function of olfactory communication in this species. The discussion will be divided into two parts. The first section will present a summary of data collected on the sternal integument and scent marking behaviour in this study. Possible functions of scent marking and mechanisms by which sternal gland odours operate will be proposed in the second section using information presented in the first, and data from other studies.

6.1. The sternal integument and scent marking

Sexual dimorphism in odour producing structures in mammal is well known (Mykytowycz 1972; Johnson 1973; Stoddart 1974, 1980b; Thiessen & Rice 1976). Often sexual dimorphism is related to the presence or absence of scent glands, with the male of the species more likely to possess a particular gland than the female (eg the frontal and gular glands found in male but not female sugar gliders, *Petaurus breviceps* (Schultz-Westrum 1965, 1969; Stoddart & Bradley 1991)). Sexual dimorphism can also occur when both sexes possess the same odour producing gland, such as occurs with the sternal gland of the brushtail possum. In this situation the sexual dimorphism is expressed in the level of development of the glandular tissue and/or in the use of secretions produced by the gland.

A number of important differences in the morphology and histology of the sternal integument and scent marking behaviour of male and female brushtail possums have been highlighted in this study. A summary of the findings for males and females are presented in Tables 45 and 46 respectively. Data for immature animals have not been presented here, as in previous chapters it has been shown that the sternal integument and sternal gland scent marking behaviour are characteristics of sexually mature animals. The discussion will focus on mature brushtail possums only.

For mature male possums the various pieces of data collected in the field and roadkill studies show the same general pattern. During the pre-breeding and breeding seasons, scent marking behaviour is at its highest level, the amount of staining on the fur around the sternal gland is at its greatest and elements of the holocrine sebaceous tissue in the sternal integument show their greatest development. These observations correspond with the onset of oestrus in mature females and the period of consort relationship between males and females (Winter 1977). Scent marking by males was observed to cease once mating had occurred and females had young in their pouches. During this post-breeding period the lowest levels of sternal gland scent marking in males were observed. Similarly, sternal staining was at its lowest level and both glandular tissue types, that is the holocrine sebaceous and the sudoriferous apocrine tissues, showed their lowest levels of development. During the dispersal period when young from the autumn breeding season were becoming independent the level of scent marking and sternal staining increased in

males. Unlike the pre-breeding and breeding periods, however, the sudoriferous apocrine tissue rather than the holocrine sebaceous showed increased development.

The situation in the mature female possums is not as clear as for the mature males. There were a number of difficulties with the data available for the histological study of roadkills, including small sample sizes and problems accurately classifying the females into reproductive groups (see *Chapter 2, §2.2.3.2 Female maturity and reproductive state)*. Observations for female possums may be further complicated by variations between females with different breeding success — this hypothesis will be discussed in *§6.2.1 (Functions in females)* which follows. Despite these problems a number of observations can be made. Firstly, sternal gland scent marking was greatest among females with young. This did not, however, correspond with the greatest amounts of sternal staining or the highest levels of development in either glandular tissue in the sternal integument. Staining of fur around the sternal gland was greatest in females in oestrus. This corresponded with the greatest development of sudoriferous apocrine tissue components, but was not associated with a high level of scent marking behaviour. The greatest amount of development in the holocrine sebaceous tissue of the sternal gland was observed in anoestrus females. Unlike males this did not correspond with the highest levels of sternal staining or scent marking.

Table 45. Seasonal differences in sternal integument morphology, histology and sternal gland scent marking behaviour: a summary for mature male brushtail possums.

	Pre-breeding (Jan, Feb, Mar)	Breeding (Apr, May, Jun)	Post-breeding (Jul, Aug, Sep)	Dispersal (Oct, Nov, Dec)
MORPHOLOGY				
Sternal Staining Index	✓✓✓	✓✓	✓	✓✓
HISTOLOGY:				
Holocrine sebaceous tissue	✓✓✓	✓✓✓	✓	✓✓
Sudoriferous apocrine tissue	✓	✓	✓	✓✓✓
BEHAVIOUR:				
Scent marking	✓✓✓	✓✓	✓	✓✓

Key:		
	✓✓✓	greatest level of development/activity
	✓✓	intermediate level of development/activity
	✓	lowest level of development/activity

Table 46. Reproductive differences in sternal integument morphology, histology and sternal gland scent marking behaviour: a summary for mature female brushtail possums.

	Anoestrus	Oestrus	With Young
MORPHOLOGY			
Sternal Staining Index	✓✓	✓✓✓	✓✓
HISTOLOGY:			
Holocrine sebaceous tissue	✓✓✓	✓	✓✓
Sudoriferous apocrine tissue	✓✓	✓✓✓	✓✓
BEHAVIOUR:			
Scent marking	✓	✓	✓✓✓

Key:		
	✓✓✓	greatest level of development/activity
	✓✓	intermediate level of development/activity
	✓	lowest level of development/activity

From this summary, differences are apparent in the expression of sternal staining and the relative development of the underlying glandular tissue of the sternal integument between the sexes. In males the largest amount of sternal staining corresponds with the greatest development of the holocrine sebaceous tissue. In females sternal staining is greatest when sudoriferous apocrine tissue components show their highest level of development. Further evidence for gender differences in the morphology and histology of the sternal integument come from other studies. Superficially the appearance of the sternal region in males and females differs. Bolliger and Hardy (1944) describe the hairs covering the sternum in males as being "more brilliant" than in females and the size of the staining from secretions from the gland had been shown in this study and others to be larger in males. Bolliger (1944a & b) demonstrated that development of the sternal integument, the brown colour of sternal hairs and the production of secretion which stains the sternal hairs and surrounding fur was under the control of sex hormones, particularly testosterone. It has also been demonstrated that the chemical composition of secretions produced by the sternal gland tissues is different in males and females (see Biggins 1979 and Salamon 1994, 1998).

All these observations provide evidence that although brushtail possums of both sexes have a sternal integument producing odorous secretions that are deposited using scent marking behaviour, a level of sexual dimorphism in their gross morphology and histology exists. The results suggest that the two glandular tissue components of the sternal integument, that is the holocrine sebaceous and sudoriferous apocrine tissue, operate differently in each sex. Sexual dimorphism is also apparent in the deposition of secretions produced by the sternal gland. The following section will discuss the differences in the use and possible functions of sternal gland secretions in males and females.

6.2. Function of scent marking and mechanism of sternal odour action

In this section possible functions of sternal gland odours and scent marking in the brushtail possum with respect to previous research and the findings identified in the previous chapters of this study will be discussed. The functions for each gender will be considered separately. This will be followed by a consideration of the possible mechanisms of odour function.

6.2.1. Function in male possums

Sternal gland development and scent marking behaviour in males can be divided into three distinct seasonal groupings. The first occurs in the pre-breeding and breeding periods. At this time scent marking and the holocrine sebaceous components of the sternal integument are at their highest levels. In the second phase, during the post-breeding period when females have young in the pouch, the development of both sternal gland tissues and the rate of scent marking is at its lowest. The third period corresponds with the dispersal of juveniles born in the previous autumn breeding season. During this time the sudoriferous apocrine components of the sternal integument show greater development and scent marking increases.

Observations from other studies of mammals suggest that the increase in scent marking by males in the breeding season may have a number of functions. Firstly, deposition of odours may function to familiarise females with a potential mate before mating takes place. Courtship routines, which often involve odours, are used by a number of usually solitary

species to lower the level of aggression in females toward males that they usually have no contact with. A second possibility is the priming role of odours in mating. In a number of eutherian species, for example, mice (Whitten 1956; Vandenbergh 1969) and prairie voles *(Microtus ochrogaster)* (Carter *et al* 1980) odours from males are known to influence oestrus cycling in virgin and sexually experienced females. Among marsupials there is some evidence that the odour of males also influence the oestrus cycle of females (eg. Gray short-tailed opossums, *Monodelphis domestica* (Fadem 1985, Fadem & Rayve 1985). A third function for odours may be to deter other males in the area or adjacent habitats from entering the area and mating with the resident females.

Bolliger and Hardy (1944) suggested that odour and colour of the sternal gland of males in the pre-breeding and breeding period served to attract females. They also suggested that males may mark den sites to "guide the prospective partner". Reports of male odours being attractive to females has been reported in other species such as mice (Bronson & Caroom 1971) and meadow voles *(Microtus pennsylvanicus)* (Ferkin & Seamon 1987). Winter (1977) stated that is was unlikely that odour from the sternal gland deposited by males in consort relationship with females acted as an attractant. This conclusion was based on a number of observations. Firstly, sternal gland scent marking did not appear to be directed at the female, with the majority of marks being made behind the female as the male followed her. Secondly, marks that females did encounter did not appear to elicit any response. Although these observations and knowledge about the onset of oestrus in female possums (see Tyndale-Biscoe 1984 and Tyndale-Biscoe & Renfree 1987) rule out a priming function for odours produced by males, the possibility that odours have a role to play in the relationship between males and females during the consort period should not be dismissed.

It is known that the purpose of the consort period is to decrease aggression in the female when mating takes place (Winter 1977). Winter observed that mating between a male and female without a prior consort period was much more aggressive than between pairs that had engaged in a time of consort. It is probable that sternal odours do play a role in decreasing aggression in the female. During the consort period the male effectively saturates the home range of the consort female with his odour. Although a female does not overtly react to the scent marks left by a male, she is probably aware of his scent. The role of odours in decreasing aggression during mating has been shown in a number of species. In most instances female odours have been observed to reduce aggression in the male (eg mice (Mugford & Nowell 1970); see review in Ebling 1981).

Odours produced by the sternal gland prior to mating may function to deter rival males. Agonistic encounters between males have been shown to be higher during the breeding season (Kean 1967; Winter 1977) and the majority took place in the presence of a female who was usually in oestrus (Winter 1977). Where a dominance hierarchy exists among males the use of odour rather than agonistic display and fighting would eliminate the possibility of injury. Furthermore a male that does not have to leave his consort female to fight has less chance of another male mating with her in his absence. Winter (1977) suggested that as well as decreasing aggression in females, the consort period enables males to establish "ownership" of a female. If a female is being accompanied by a male his visual presence may reduce the likelihood of another male mating with her when she comes into oestrus. When visual contact between a resident consort and a potential rival are not possible, scent marks may serve to alert other male that the area is occupied and that resources, such as oestrus females, are already "owned". Winter suggested that odours deposited by the consort male are superimposed on the females odour as he follows her around their overlapping ranges. Although Winter did not report any over-marking of female scent marks by males, the deposition of secretions by a male as he follows a female may cover any passive odour trail left by the female. This action may act to mask odours produced by the female that indicate she in coming into oestrus which in turn may lessen the chances of a rival male mating with the female. Possible mechanisms for the action of odours in deterring rivals will be discussed in *§6.2.3 (Mechanisms of odour function).*

The second seasonal phase of scent marking which occurred during the post-breeding period and corresponded with the time when females were carrying young in the pouch is characterised by a lack of marking and a low levels of sternal gland development. It is known that the majority of females give birth during the autumn breeding season (Bolliger 1940; Dunnet 1964), therefore during the post-breeding period males will not be acting as consorts to females. Furthermore if females have young there is no need for males to actively deter potential rivals for females from entering their home range. Effectively the resource they were protecting, that is a female approaching oestrus, is no longer present. If marking during the breeding season is related to decreasing aggression in females and deterring potential rivals it is not unexpected that marking is almost non-existent during the post-breeding period. That scent marking and sternal gland development are low during this period may also be related to the low level of transient individuals moving around in search of suitable habitat. In this study only one transient male was captured during the post-breeding period. If, as suggested by Winter (1977), scent marking functions to protect resources such as den and feeding trees, the low level of new arrivals during the post-breeding period would correspond with a low level of marking.

The third phase of sternal gland development and scent marking is seen during the dispersal phase. Male possums are thought not to possess exclusive territories (How 1972; Crawley 1973; Winter 1977) and there is no evidence from this study or others (eg Winter 1977) that scent marks are deposited around the boundary of home ranges. Rather males have overlapping home ranges that contain areas that are exclusive to other individuals of the same sex and age (Winter 1977). Winter reported that scent marks were concentrated in these exclusive areas and that focal points for marking included den sites and feeding trees. Marking at this time of the year is most likely related to the protection of resources such as den trees and feeding trees.

The pattern of dispersal among juvenile possum shows gender differences with juvenile males tending to dispersed further from the maternal home range than females (Dunnet 1964; Winter 1977; Ward 1985). Differences in the pattern of dispersal may be due in part to different levels of aggression towards juveniles of each sex. Winter (1977) did not observe any aggression by adult males towards juvenile females. Among juvenile males, however, Winter proposed that adult male aggression was partly, if not wholly, responsible for their dispersal. It is probable that sternal gland odours are involved in the dispersion of juveniles. Biggins (1979) reported in experiments with captive male possums that sternal gland scent marking was high during agonistic encounters between males. Most of the sternal marking was performed by the dominant male, with subordinate individuals depositing scent from the paracloacal glands more often. In agonistic encounters in the field between juveniles born in the area and resident adults its is likely that the subordinate juvenile come to associate odours deposited by the older, more dominant males with aggression. Detection of these odours when the resident male is not present may deter the juveniles from remaining in any area that contains the resident's scent.

The dispersal of juvenile males results in a pool of transient individuals without home ranges. Success in establishing a home range is believed to be dependent upon finding a suitable den tree that is not used by a resident male or female (Winter 1977). Juvenile males are known to be recruited into populations (Clout & Efford 1984) and it has been shown that younger males are tolerated within the home range of older, established males (Winter 1977). The maintenance of the overlap between younger and older males may also involve odour. Aggressive encounters between juveniles of unknown origin and resident males have been reported (Winter 1977) and these probably function to establish a hierarchical relationship between the males. Again odours from older dominant males encountered by younger subordinate males when the older male is not present may operate as a deterrent and serve to maintain the hierarchical relationship without ongoing agonistic interactions.

It should be noted that Winter (1977) did not report a high level of sternal gland scent marking by males in the dispersal period. Between October and December sternal gland

scent marking was at its lowest in males at Winter's site. The discrepancy between the observations in this study and Winter's may be related to differences in the type of dispersing individuals at the two sites. In Winter's study dispersing individuals were juveniles. In this study most of the individuals caught during the dispersal period were transient mature males, rather than juveniles. Although Kean (1975) has reported that dispersing juveniles are not often captured, it was known that there were very few juveniles at this site. In both years of observation only a small number of females successfully reared their young to independence. As outlined earlier it is known that young males are tolerated in the home range of established male, but areas within the established male's range are exclusive to male of similar age and status. Furthermore, the ability to establish a home range is dependent upon finding a suitable den tree that is not in used by a resident male or female (Winter 1977). The low level of marking at Winter's site may reflect a greater availability of unused resources and/or tolerance of young males entering the area. It is likely that the differences in population density at different sites are reflected in the level of marking during the dispersal period. The higher level of scent marking in this study may be a reflection of the limited resources such as den and food trees. Support for this idea is provided by the observation that none of the transient males captured during this dispersal period were caught again. Scent marking in this situation may be functioning in a resource protection role that operates by indicating to transient adult males that another established male already occupies the area. The inability of transient to become established in area already occupied is further supported by the observation that possums displaced by habitat destruction are unable to become re-established in adjacent areas that are already occupied by conspecifics (How 1972). Further evidence that scent marking has a resource protection role comes from observations in Winter's (1977) study that den and food trees are often marked, and observations in this study that a large percentage of scent marks are made on or near trees and traps.

The suggestion that there are differences in the function of sternal gland scent marking by males during the pre-breeding/breeding period and the dispersal phase is supported by two observations. The first comes from examination of the histology of the sternal integument in this study. The observation that sudoriferous apocrine tissue development is greatest during the dispersal period and holocrine sebaceous during the pre-breeding/breeding seasons suggest that a different message is contained in odours produced in each season. This is upheld by analysis of the chemical components and relative concentrations of chemical in male sternal gland secretions which have been shown to differ between the two times of the year (see Salamon 1998).

6.2.2. Functions in females possums

Winter (1977) reported that sternal gland scent marking by females occurs predominantly between the first and last times a young is seen riding on its mother's back. Joeys begin to ride around on their mother's back at approximately 4-5 months of age (Dunnet 1956, 1964; How 1972) and continue to do so for 1-2 months before becoming independent. This shows some correlation with the observation that the sternal region in females becomes increasingly stained and moist 2-3 months after the birth of the young (Bolliger & Hardy 1944). Winter (1977) observed that scent marking by females with young on their back coincided with increasing antagonistic behaviour of the female towards her young and marked the beginning of the breakdown of the mother-joey bond. It is interesting to note that marking was only seen in females with female offspring. It is probable that scent marking in this context serves to establish and maintain a hierarchy between the female and her daughter. Support for this hypothesis is provided by the observation that females are more likely to be recruited into the population than males (Clout & Efford 1984) and that the home range of young females are often contained within or overlap significantly with the maternal home range (Dunnet 1964; Winter 1977). Young females that remain within the maternal home range are in direct competition with their mother for den sites and other

resources, although the existence of natal philopatry in this solitary species means that a related female is less of a threat than unrelated females because they share common genes (see Waser & Jones 1983). Even though Winter (1977) did not observe any overt response by juvenile females to scent marks made by their mother it is possible that the odours become associated with the increasing aggression displayed by the mother during this period as the mother-young bond begins to break down. Once the bond has been completely broken odours from the older female may function to maintain a dominance hierarchy between the mother and her now adult offspring.

In this study the use of spool-and-line tracking prohibited observation of females carrying young on their back. Observations made at all other times of the reproductive cycle, however, revealed that females at this site began marking soon after the appearance of the young in the pouch and continued to do so while the young remained in the pouch. Winter (1977) did not report a high level of marking among females with pouch young. There are a number of possible reasons for an increase in scent marking by females once they are carrying pouch young. The first may be to advertise to males that they already have already mated successfully and have a young. In a number of mammalian species males have been demonstrated to be able to discriminate between odours of oestrus, anoestrus and pregnant females (see review in Eisenberg & Kleiman 1972) and that females in oestrus are more attractive to males (eg rhesus monkeys (Bonsall *et al* 1978); golden hamsters, *Mesocricetus auratus* (Johnston & Rasmussen 1984); meadow voles, *Microtus pennsylvanicus* (Ferkin & Johnstone 1995)). Male possums acting as consorts have certainly been observed to investigate areas where females have been sitting, presumably to determine her reproductive condition (Winter 1977). It is possible that once females have mated successfully they may mark when they come in contact with a male enabling him to inspect the scent mark and determine the her reproductive state. It has been observed that aggression of females towards males increases once they have a pouch young (Jones 1921).

It is more likely that the purpose of scent marking by females with pouch young is related to the protection of resources during the winter months to provide shelter and food for the rapidly growing pouch young. Pouch young are carried through the winter months and having an adequate den to shelter in may be vital for successfully rearing a young. The mean daily minimum temperature during winter at the Mt Morrison State Forest are between 3.3-4.0°C and the mean maximums between 13.1-14.0°C[3]. These temperatures are much colder than those experienced by possums at Winter's site in Queensland. The winter months are also a time of lower food availability, compared to autumn and spring when fruit and flowers are abundant (Fitzgerald 1984). During 1993 and 1994 food may have been even more limited than in other years due to very low rainfall in both years at the site.[4] Among the five females for which there were adequate reproductive data there was a high loss of young during the pouch stage. Previous studies have not reported high mortality of young in the pouch (Dunnet 1964; How 1972). Mortality at this stage of life is usually very low, with the highest rate occurring when juveniles disperse from the home range (Dunnet 1964; How 1972; Winter 1977). Of seven young born to females during two autumn breeding seasons only two survived past pouch life and were observed riding on their mother's back. The remaining five young survived for between two and four months only. There were two obvious differences between the successful and the unsuccessful mothers. Firstly, the females who reared their young past the pouch life stage maintained and actually increased their body weights (250-300g) over the winter months when the young was in the pouch. The unsuccessful females all lost considerable amounts of weight, between 350 and 1250g, which was between 12% and 43% of their pre-pouch young weight. A similar observation of the presence of "poor conditioned and fat

[3] Temperature data are from the nearest temperature weather station at Orford (Latitude 42°33'06"S, Longitude 147°52"39"E, Elevation 15m). Temperatures at the Mount Morrison State Forest (elevation 300m) in winter are probably lower than those stated. Data provided by the Climate and Consultancy Section of theTasmania and Antarctica Regional Office of the Bureau of Meterorology.

[4] Based on rainfall data from the Nugent (Twilight Valley) weather station (Latitude 42°43'00"S, Longitude 147°45"02"E, Elevation 320m. Data provided by the Climate and Consultancy Section of theTasmania and Antarctica Regional Office of the Bureau of Meterorology

opossums" in the same habitat has been reported in New Zealand (Kean 1971). The second difference was that the successful females had higher rates of sternal gland marking when carrying young than the unsuccessful females. A third point of interest is that the two successful females had overlapping home ranges. In the first year of the study one female successfully reared a young while sharing an area with the other female. The second female was a juvenile in the first year of the study and was possibly the mature females' offspring from the previous season. The first female disappeared from the site and the second female successfully reared her own young in the same area the following year.

It is possible the conditions at this site were somewhat harsher and more difficult for survival than at Winter's site, in terms of needing shelter from low winter temperatures and with respect to the availability of food. High mortality has been reported in possums with poor shelters during winter in New Zealand (Pracy pers. comm. in Kean 1967). Kean (1971) also reported that shelter and food are not equally shared among possum. To ensure survival of offspring, females with adequate resources would need to employ a method to protect those resources from females without adequate supply. The high level of scent gland development and sternal gland marking seen in females with pouch young, particularly those able to rear young successfully, may be a reflection of the need to protect limited resources. The lack of marking by females with pouch young in Winter's study may reflect greater availability of resources required for successfully rearing young at his site. As in male possums the observation that resources such as den sites, food trees (Winter 1977) and traps (this study) are scent marked provides support for the theory that scent marking has a resource protection role.

The importance of adequate resources for successfully rearing young has been highlighted in a study of Eurasian red squirrels (see Wauters & Dhondt 1989,1992 and Wauters *et al* 1995). This species shows some interesting similarities with the brushtail possum. Mature female red squirrels defend intrasexually exclusive territories against other females, with subadults and yearling females behaving as "floaters" or settling in home ranges on the edge of adult female core areas (Wauters & Dhondt 1992). The territories are defended during the year, but particularly in the breeding season (Wauters *et al* 1995) and only territory-holding females produce offspring (Wauters & Dhondt 1989). It was observed that occasionally females shift their territories. Shifting was seen in females with territories that were poor in food resources and only occurred if the new territory contained more food than the old one. The consequence of the change in territory was an increase in reproductive rate (Wauters, Lens & Dhondt 1995).

In summary it is apparent that sternal gland scent marking in male and female brushtail possums operates on a number of levels. Firstly, in both sexes odours appear to be used to establish intrasexual dominance hierarchies between older resident animals and younger juvenile and subadults that were born in the area. Secondly, odours are being used to protect resources. In females den sites and food trees required protection during the post-breeding period. In males den trees and food trees may be the initial focus during the dispersal period when transient males (immature and mature) enter their home range. In the long term preventing new males from establishing themselves eliminates potential rivals for females in the breeding season. During the pre-breeding and breeding periods the main focus for resource protection is females approaching oestrus. The way in which odours may operate in the establishment of dominance hierarchies and in the protection of resources will be discussed in the following section.

6.2.3. Mechanisms of odour function

Evidence presented in the previous sections strongly suggests that a major role for olfactory communication, particularly sternal gland secretions, in the brushtail possum is the protection of resources. If this is so, how do odours from scent glands operate to protect a resource, whether it is a den site, a food source or a female approaching oestrus?

Winter (1977) and Biggins (1979) have suggested that odours from the sternal gland may contain information about the gender, age, social status and individual identity. Evidence of differences in scent gland size and structure, quantity of secretion, chemical composition of secretions and scent marking behaviour between possum of different gender, age, social status and reproductive state at different times of the year from this study and others (see Bolliger & Hardy 1944, Winter 1977; Biggins 1979: Salamon 1998) support this hypothesis. In many other mammalian species differences in scent glands and marking behaviour have been demonstrated for different individuals within a species. Furthermore it has been shown that individuals are able to differentiate between individuals of different sex, age, social and reproductive status on the basis of odour (Eisenberg & Kleiman 1971; Brown 1979). The response of a conspecific to an odour may also show variation depending upon the sex, age, social and/or reproductive state of the individual. It is important to remember that social context, previous experience and relationships between individuals can all influence the response on an individual to the odour of a conspecific (Müller-Schwarze 1974).

Whatever information a sternal scent mark may contain, the message of resource ownership probably operates in three ways in the brushtail possum — through dominance hierarchies among adjacent and overlapping individuals, through "scent matching" and "competitor assessment" by potential intruders and rivals, and through "confidence boosting" and "reassurance" of the scent marking resident.

Although the brushtail possum is a solitary species a hierarchy exists among adjacent individuals of the same gender, based on the age of individuals and centred on resources including den and food trees and oestrus females (in the case of males). The establishment of a dominance hierarchy between mature resident animals and younger individuals appears to be the result of agonistic encounters. Among females there is evidence that mothers begin to establish dominance over female offspring once they leave the pouch by gradually increasing their level of aggression toward the young (Winter 1977). As the level of aggression towards the young increases the level of sternal gland scent marking by the mature female also increases. While aggression may serve to establish the hierarchy between the female and her offspring, odours are probably used to maintain it. It is probable the young come to associated odours with aggressive behaviour which in turn leads to avoidance of scent marked areas once the young has become independent. In this way the now mature offspring is able to remain within the maternal home range by avoiding core areas that have been scent marked. Among mature and juvenile males it is probable that the establishment and maintenance of dominance operates in a similar manner. In males, however, the majority of juvenile males born in the area disperse, with males of unknown origin moving into the area (Dunnet 1964; Winter 1977; Ward 1985). Aggression between these juvenile males and resident males has been reported (Winter 1977) and probably serves to establish a dominance hierarchy. As with the females, juvenile males may come to associate odours from scent marking by the older more dominant male with aggression and avoid areas where such odours are found in the future. Because scent marks are concentrated around particular resources and at particular times of the year it is possible for the home range of younger males to overlap with older individuals as long as they avoid the scent marked core areas.

Odours have been demonstrated to play an important role in the establishment and maintenance of dominance hierarchies in many mammalian species (Ralls 1971). A range of physiological and behavioural differences between individuals of different dominance

status exist (Ralls 1971), and among males these have been linked to levels of testosterone (Brown 1979). With respect to scent glands, scent marking and odours, dominance status may be reflected in differences in the size of scent glands, differences in the quantity and quality of the secretion produced by the gland and/or differences in the frequency of scent marking (Brown 1979). Differences between older, more dominant individuals and younger subordinates have been shown in all these parameters in the brushtail possum.

In situations where there has been no visual or physical interaction between a resource holder and a potential rival, odours may provide an intruder and potential rival with information about the marker. This is analogous to the situation that may occur when transient and dispersing individuals from other areas enter the home range of a possum. The use of odours for territorial defence is well known (Hediger 1949; Mykytowycz 1972; Johnson 1973; Müller-Schwarze 1983; Gorman 1984). Recognition of boundaries using odour has been demonstrated, for example, in mice (Harrington 1974), black-tailed deer (Müller-Schwarze 1971) and lemurs (Millhollen 1986). There is no evidence to suggest that scent marks are located around the boundary of home ranges in the possum (Winter 1977; this study Chapter 4). And in a series of experiments with captive male possums Biggins (1979) demonstrated that individuals did not avoid areas marked by conspecifics. Even in species where boundary marking is used extensively there is not much evidence to suggest that scent marks actually deter an individual from entering a marked area (Gosling 1982). Brushtail possums do concentrate marking at important areas in their home range, such as den sites and feeding trees, and males acting as consort mark in the vicinity of oestrus females (Winter 1977). In situations where individuals were known to each other Winter (1977) reported that subordinate males would investigate scent marks deposited on tree bases by consort males following females during the breeding season. The scent mark appeared to deter the subordinate male from climbing into the marked tree that may or may not have still contained the consort pair. In other situations there is little evidence of any overt response to scent marks.

It has been suggested that rather than deterring potential rivals, resource holders mark to provide intruders with a means of competitor assessment (Gosling 1982, 1989; Gosling & McKay 1990). This theory works on the basis of scent matching. Odours do not as such deter an individual from entering an unfamiliar area, but rather provide the intruder with information about the owner. If the intruder and owner of the area encounter each other the intruder is able to recognise the owner based on a comparison of odours from scent marks and the odour of the individual. In a series of experiments, fighting behaviour of male mice when on a scent-marked substrate that matched the odour of an opponent was compared to their behaviour on a substrate that did not match (Gosling and McKay 1990). When the scent matched the opponent, fighting was delayed and the rate of fighting was lower. Gosling and McKay (1990) suggest this demonstrates that male mice are able to assess potential opponents by comparing their odour with scent marks in the vicinity. The result is that competitors are less likely to fight when they meet the owner of a resource, effectively reducing the cost of resource protection for the resident because they do not have to engage in fighting which may result in injury. If odours contain information about sex, age, and status an individual may be able to assess the resident before entering the area.

Related to the idea of competitor assessment is the notion that odours work by "boosting the confidence" of the marker (Ewer 1968; Mykytowycz 1972, 1974; Johnson 1973; Shorey 1973; Brown 1979; Müller-Schwarze 1983). Whereas odour in competitor assessment operates to provide the potential intruder with information about the resident, odours in the confidence boosting-reassurance theory operate as much for the marker as for the intruder. It has been suggested that animals behave freely and participated in breeding activities only in areas where their own odours prevail (Mykytowycz 1972, cited in Mykytowycz 1972). Captive male possums have been observed to actively explore and scent mark clean, novel cages. The level of scent marking and investigation decreases as the area becomes saturated with odours (Biggins 1979). Furthermore, it is well known that resident individuals are more successful than intruders during agonistic encounters

(Eisenberg & Kleiman 1972). This has been demonstrated in the brushtail possums. In encounters between captive male possums resident individuals were consistently more successful than intruders (Biggins 1979). The role of odour in the outcome of encounters has been demonstrated in situations were it was possible to reverse the usual outcome by placing the victor in an area with unfamiliar odours (Biggins 1979). Success of individuals appears to be due partly to enhanced confidence of the resident animals, with respect to deterring intruders by engaging in aggressive interaction when on familiar ground. This contrasts with a decrease in confidence leading to lower motivation to engage in agonistic behaviour in individuals in unfamiliar areas. Levels of aggression between individuals decreases once they become familiar with one another. In possums, unfamiliar individuals show more aggression toward each other than familiar individuals (Biggins 1979). The importance of odour in recognition was demonstrated in experiments with possums rendered temporarily anosmic, with unfamiliar pairs of possums displaying less aggression than would have been expected (Biggins 1979). The result of encounters between unfamiliar individuals is the establishment of a dominance hierarchy. Intruders of a similar age and size to a resident, such as the transient males captured in this study, are potential rivals for resources. Having the advantage of being on familiar home ground and therefore being more likely to dominate in aggressive interactions, residents are able to exclude closely matched rivals. Rivals would only be able to establish themselves in areas were resources were not limiting, such as areas left vacant by another animal or areas where resources are abundant and densities low. By using odour to recognise areas that are occupied, and limiting movement into marked areas a pattern of mutual avoidance and distinct home ranges results.

The importance of odour in the life of this nocturnal marsupial cannot be disputed. Data from this study and others suggest that both male and female possums possess sternal glands that they use to mark their home ranges particularly for the protection of resources. Scent marking is involved in the establishment of intra-sexual dominance hierarchies, with older, more dominant individuals having greater access to important resources which enable them to breed more successfully than younger subordinate individuals. Odours are vital for the maintenance of hierarchies and home ranges that serve to protect important resources required for reproduction and the survival of the species. Although this study has focused on the sternal gland it should be remembered that possums have a range of odour producing structures. Further research integrating biological and ecological knowledge with information about the morphology, histology and behavioural aspects of scent glands will no doubt increase understanding of the importance of olfactory communication in the brushtail possum.

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